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DIURNAL FLUCTUATION IN ROOT PRESSURE[1]

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(WITH FOUR FIGURES)

Diurnal cycles in root activity under constant conditions seem to have been first described by HOFMEISTER in 1862 (**7**). Other investigators have confirmed his findings (**1, 11**), but in recent years it has been concluded that cycles in pressure and exudation were due to temperature fluctuations (HEYL, **5**). On the other hand, studies in this laboratory (**9**) have substantiated HOFMEISTER's original results, and WHITE (**10**) has reported a cycle in exudation of excised tomato roots (though he did not control the conditions under which he was working). Complete proof of the existence of autonomic cycles has recently been obtained by the author with plants grown and tested under controlled conditions (**4**).

Several theories have been developed to explain the general phenomena of root pressure and exudation. For the purpose of this discussion it is sufficient to point out that osmotic mechanisms have generally been used. SABININ (**8**) holds the view that a simple osmotic mechanism is sufficient.

The general problem is confused by the controversy over the parts played by the phloem and xylem in translocation and root pressure. Since these measurements extended over periods of several days it seems probable that sieve tubes were plugged (CRAFTS, **3**) and that the phloem played little part directly in maintaining the observed pressures. Here it is assumed that the hydrostatic-pressure system involved is the xylem-manometer system.

In these studies, plants (*Helianthus*) were grown in culture solution with forced aeration, in a greenhouse. The solution concentration was low, $\frac{1}{4}$ H— (see explanation later) and typically not renewed, so that the plants were vigorous and healthy at the time of experimentation and were comparable to the "low salt plants" of HOAGLAND and BROYER (**6**), with root systems capa-

[1] The experimental work on which this paper is based was carried out at the University of California (at Berkeley) through the cooperation of the Department of Botany and the Division of Plant Nutrition, during the summer months of 1937.

ble of very active absorption. On several occasions such root systems maintained pressure for 14 days, indicating that they were quite healthy, had large food stores, and were "starved" only in the technical sense of having a low salt content.

A fairly satisfactory experimental technique has been developed: Plants were grown in two-quart jars fitted with sintered glass aerators. Small bore (1-mm.) u-tube manometers were built so that they might easily be fastened to the side of the jar with heavy rubber bands (fig. 1). The small

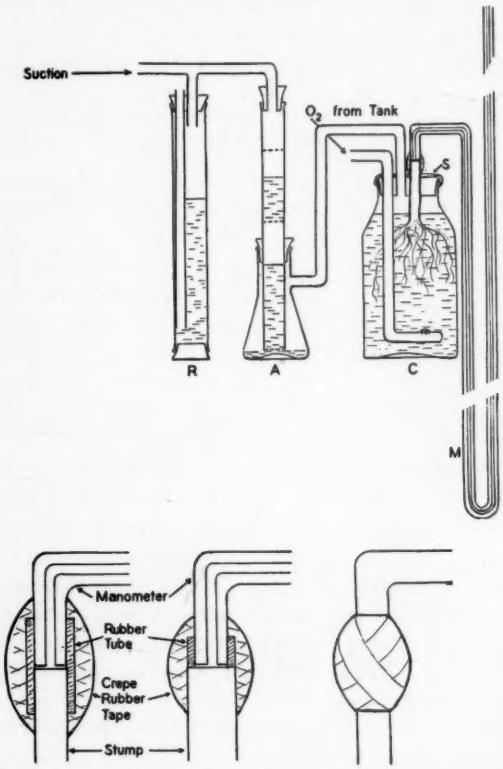

FIG. 1. Apparatus used to measure root pressure and respiration; R, regulator on suction line; A, absorption column; C, culture vessel (in which the plant was grown); S, paraffine seal; M, small bore mercury manometer.

bore and double action of the u-type manometer gave a convenient method of measuring pressure changes with only small change in volume of liquid in the xylem-manometer system (0.004 cc./cm. Hg). Manometers were attached to squarely cut stumps with the aid of rubber tubing and crepe rubber tape

("Sterilastic tape" carried by most drug stores). Such attachments could be made to withstand more than two atmospheres of pressure and resulted in a minimum of crushing or injury to stems. Except as designated, all solutions were balanced nutrient solutions, HOAGLAND'S solution (KH_2PO_4 0.001M; $MgSO_4$ 0.002M; KNO_3 0.005M; Ca $(NO_3)_2$ 0.005M) served as a base. Concentrations are expressed as ¼ H, 1 H, and 4 H, representing fractions or multiples of the original solution. During the growing period additions of iron and supplementary solution (boron, etc.) were made as needed. Plants grown in a greenhouse were transferred to a thermostatically controlled darkroom, and pressures were recorded at short intervals (usually 3 hours) under constant conditions of light, temperature, and aeration.

One of the most pronounced effects observed was a diurnal fluctuation in pressure which could not be correlated with any change in the environment of the plant during the test period. Careful study of thermograph records showed that there were slight shifts in temperature during extended periods but no diurnal fluctuation could possibly be read into the record for many of the days when root pressures showed such fluctuations.

When it became evident that root pressure did not reach an equilibrium under experimental conditions, considerable effort was made to secure as constant an environment as possible. Tanks of compressed oxygen were used to furnish a uniform supply of oxygen for the aeration of solutions. Artificial light at several intensities was used and remained as constant as commercial electrical service. These improvements did not measurably affect results and it is concluded that these plants were capable of maintaining a diurnal cycle in root pressure which was not directly related to current conditions but possibly controlled by a physiological cycle set up during the growth period in the greenhouse.

Experimentation

Considerable variability was observed and not all plants showed such fluctuations (of special interest was a group of plants which had symptoms probably resulting from boron deficiency and which did not show a diurnal cycle). Data from three experiments are presented to illustrate some of the typical responses observed:

EXPERIMENT I

"Pure line" seed obtained from Cornell (S-24–150) was used. Plants were selected twice during their five-weeks growing period, and were quite uniform and vigorous. Setting up of the experiment began at 3 P.M. The solutions were changed to ½ saturated $CaSO_4$ (*i.e.* 0.005M±) and ½ H, manometers attached, and aerators connected (tank O_2), by 7 P.M. Absorption towers for CO_2 were connected at 8 P.M. thus allowing a full hour for the solutions to come into equilibrium with the gas stream (the rate of gas flow was well above one liter per hour). Pressures were recorded every three

hours and respiration determined for six-hour (later twelve-hour) intervals. Data are presented graphically in figure 2, each curve representing the average

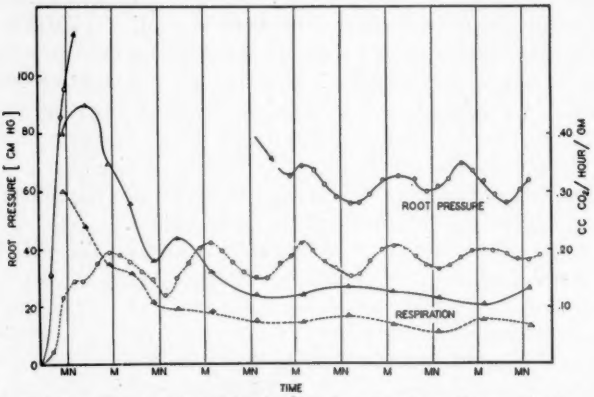

FIG. 2. Root pressure and respiration of 5-week-old sunflower plants. Solid lines represent averages obtained in balanced nutrient solution; broken lines represent averages obtained in dilute solution of $CaSO_4$.

for five plants. Values for individual plants were determined and plotted, but showed the same trends as the averages.

It is apparent from the curves that plants transferred to a fresh dilute, but balanced, nutrient solution respired appreciably more and developed much more root pressure than those transferred to dilute $CaSO_4$. In fact the manometers used did not have sufficient capacity (1 meter Hg) to measure the pressure produced by four of the five plants, and no significant measurement was made for a period of about forty-eight hours while mercury was being forced back into the manometers and allowed to come to equilibrium again. Subsequent readings may have been affected by the loss from the root systems of a considerable volume of exudate immediately following the "blowing out" of the manometers. Even with this treatment a fairly distinct diurnal fluctuation is evident in the root pressure values. Plants in dilute $CaSO_4$ had lower root pressures than those in complete nutrient solution and they maintained a diurnal fluctuation well into the sixth day. In neither case did the plants show a consistent relation between root pressure and respiration. Respiration values for both groups dropped rapidly during the first twenty-four hours and then leveled off with the complete-solution group maintaining a 20 to 50 per cent. higher rate.

EXPERIMENT II

Groups of three similar plants five weeks old were treated in the following manner, five days before the experiment was set up: The first group

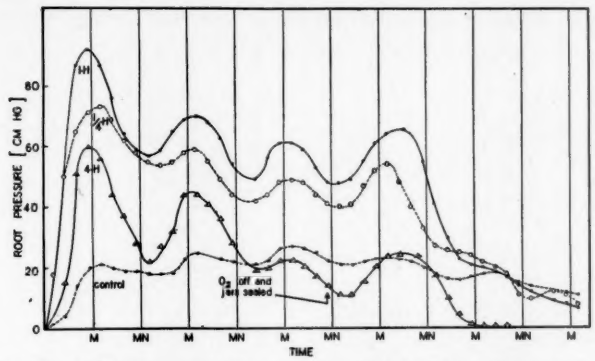

FIG. 3. The effect of concentration of balanced nutrient solution and oxygen supply on root pressure.

was left undisturbed (*i.e.*, the solution contained the unused portion of ¼ H solution in which the plants were grown), while other groups received additional nutrient salts equivalent to ¼ H, 1 H, and 4 H. Those treated with full strength solution showed the highest pressure. The untreated ones gave the lowest and most constant readings while those treated with 4 H developed a fairly high pressure but failed to maintain it. All four groups showed diurnal fluctuations.

After three days the oxygen was turned off (all groups of plants) and the jars sealed with paraffine. The curves (fig. 3) show that root pressure was definitely affected in about one day. By taking values from the previous respiration curve it was calculated that the root systems were using about 25 cc. of oxygen per day. The solution contained about 12 cc. (assuming saturation), and a small space between the solution and cork was filled with oxygen which, however, could move into the solution only very slowly, as there was no stirring, and convection currents would be greatly reduced in a thermostated room. Thus it is indicated that under these conditions *Helianthus* roots may maintain root pressure until the rate of oxygen supply is greatly reduced, but that the pressure falls when oxygen becomes too low or the carbon dioxide partial pressure too high.

EXPERIMENT III

The root pressure of four groups of three plants each were measured for two days and then additions were made equivalent to ¼ H, 1 H, and 4 H, and readings taken at short intervals (fig. 4). It should be noted that the time scale is greatly increased, and the changes are rapid. In the three cases in which additional salts were used, a sharp decline in pressure was observed; its magnitude, however, was not quantitatively related to the osmotic value of added salts. In less than two hours all pressures were rising rapidly.

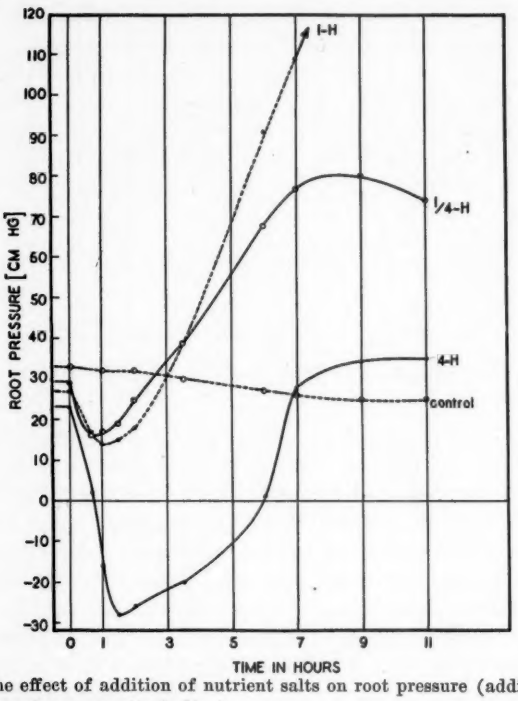

FIG 4. The effect of addition of nutrient salts on root pressure (addition made two days after manometers were attached).

In seven hours all reductions had been completely overcome. Again full strength solution gave maximum response, forcing the mercury out of the manometers. The 1 H solution used has an approximate osmotic value of 0.7 atmosphere. If we assume for convenience that its actual value is 50 cm. of Hg we see that the reduction produced by the $\frac{1}{4}$ H solution was in the same order as that expected as a result of simple osmosis but that the reductions produced by either 1 H or 4 H solution was only $\frac{1}{4}$ of that expected.

Discussion

An explanation of root pressure in terms of a *simple* osmotic mechanism seems impossible in the presence of a diurnal cycle because: first, it would be necessary to assume that solutes (organic or inorganic) were periodically released into and removed from the sap; second, since the volume of liquid in the xylem-manometer system changes in the same way that the pressure changes, it follows that water must be entering and leaving at the same time solutes do; third, to be effective osmotically, changes in solutes must occur in the same regions in which water enters and leaves.

When electrolytes are added to the external solution (exp. III) there is an apparent osmotic effect (the sudden decrease in pressure). A stimulating effect of the added salts soon overcomes this in the case of active plants and builds the pressure up again. The first response may in reality be simply osmotic. The subsequent rapid rise in pressure must be the result of water entering the xylem system, and if this water enters in response to osmotic force it must be entering while the salts are becoming continuously more concentrated.

From these considerations it seems clear that if we assume a *simple* osmotic mechanism to be responsible for these changes in root pressure we are forced to admit:

(1) that water and solutes must enter and leave the xylem system together, *i.e.*, as a solution.
(2) That such movement cannot be due to osmotic force and hence our original premise is incorrect.

Root pressure, then, is not caused simply by the difference in osmotic values of the xylem sap and the external solution, but must be caused by some other forces in the root system which control and activate this movement. Evidently the mechanism of the diurnal cycle is in some way related to the mechanism responsible for root pressure. Yet, it has long been established that under many conditions there is a relation between root activity and respiration. Several such correlations are apparent in these experiments but the diurnal cycle of root pressure was not shown to be paralleled by a cycle in respiration. If such a cycle exists, its magnitude is probably less than that of root pressure.

Changes in permeability might explain fluctuations in exudation alone (8) but will not explain pressure fluctuations in a simple osmotic system since they would affect only the rate of change of pressure and not its equilibrium values.

If, however, a complex osmotic system be postulated the above reasoning breaks down. It may be argued that with increasing pressures solutes are released into the xylem sap in localized areas (cells or parts of cells) and that water enters in response to osmotic force in the immediate vicinity, while with decreasing pressure solutes could either be reabsorbed or merely diffuse out. In this case the cycles could be explained as a shifting balance between the two opposing processes. This is not, however, a simple osmotic mechanism and it would be impossible to speak of the osmotic value of the internal solution as it would in reality consist of a whole series of concentration gradients being constantly upset by diffusion and mass flow of sap resulting from movement of water and changes in pressure. It would be exceedingly difficult to obtain direct evidence to prove or disprove such a theory.

BENNET-CLARK, GREENWOOD, and BARKER (2) and others have concluded

that in potential growing tissues there is "positive water secretion" from the protoplasm into the vacuole in addition to the "osmotic pressure of the vacuole." Whatever the mechanism of such secretion is, it is becoming increasingly clear that some such process must be involved in many phases of the movement of water in living tissues. In root pressure it seems probable that such a force may operate in either direction and will, in addition to the osmotic forces involved, determine the pressure.

It would be pretentious to list those who have in some way contributed to this brief paper, but I wish to thank them for their help, especially for criticism of the discussion.

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FOLIAR DIAGNOSIS: ITS RELATION TO THE OPTIMUM NUTRITION OF THE POTATO[1]

WALTER THOMAS
(WITH SEVEN FIGURES)

Introduction

The principles and application of the method of foliar diagnosis have been described in detail in a recent paper (**8**) using as illustrative material potato plants growing on seven plots of tier 1, section B, of the long continued Vegetable Fertility Plots of the Department of Horticulture of the Pennsylvania Agricultural Experiment Station. The plots examined in the previous paper, familiarity with which is assumed in the present paper, were:—N (plot no. 2), P (plot no. 3), K (plot no. 4), NP (plot no. 6), NK (plot no. 7), PK (plot no. 8), and NPK (plot no. 10).

The yields of tubers from the highest yielding plots previously reported, *viz.*, NK (plot no. 7) and NPK (plot no. 10), were considerably below the yields from the manure plot (no. 15) and were also below the yields from certain plots from the adjacent tier 2, which received complete mineral fertilizers also, but differed in the quantities and ratios applied. The plot (no. 15) treated with rotted horse manure at the rate of 30 tons per acre (600 lb. per 1/100-acre plot) has consistently outyielded the other plots. The plants growing on this manure plot which have shown good development throughout the season, accordingly, may be regarded as well nourished and at or nearing the optimum for the soil and climatic conditions prevailing in this region (**7**).

In this paper the relation of the foliar diagnosis of the best commercial fertilizer treatments to that of this manure plot (plot no. 15, tier 1) will be examined.

Materials and methods

PLOTS WITH TREATMENTS AND YIELDS

The experimental plan as described by MACK (**1**) is as follows:—"The area is laid out in four sections, 245.8 feet wide and 308.4 feet long ..... Each of the sections is divided into six tiers, ... The tiers are further divided into seventeen plots, the dimensions of which are 12 by 36.3 feet, and which are separated from each other by guard strips 6 feet wide. The area of the plots, exclusive of guard strips, is exactly 1/100 of an acre."

Table I lists the plots examined and discussed in this paper together with

[1] Authorized for publication as Paper no. 795 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

their fertilizer treatment and yields of tubers for the year 1935. The treatment and yields of the plots reported in the first paper of this series (8) also are given for comparison.

TABLE I

PLOT NUMBERS, TREATMENTS, AND YIELDS OF TUBERS

TIER	PLOT	TREATMENT	AMOUNT APPLIED TO PLOT	N, P_2O_5, AND K_2O EQUIVALENT	SYMBOL AND RATIO	YIELDS*
			lb.	*lb.*		*lb.*
1	15	Rotted horse manure	600†	4.62 3.08 3.78	15: 10: 12.3‡	196
2	8	Nitrate of soda Superphosphate Muriate of potash	4.0 9.375 1.666	0.6 1.5 0.8	N(1.5P)K 6: 15: 8	176
2	12	Nitrate of soda Superphosphate Muriate of potash	4.0 6.25 2.499	0.6 1.0 1.2	NP(1.5K) 6: 10: 12	174
2	16	Nitrate of soda Superphosphate Muriate of potash	8.0 13.5 3.332	1.2 2.0 1.6	2(NPK) 12: 20: 16	170
1	7	Nitrate of soda Muriate of potash	4.0 1.666	0.6 0.8	NK 6: 0: 8	163
1	10	Nitrate of soda Superphosphate Muriate of potash	4.0 6.25 1.666	0.6 1.0 0.8	NPK 6: 10: 8	162
2	4	Nitrate of soda Superphosphate Muriate of potash	6.0 6.25 1.666	0.9 1.0 0.8	(1.5N)PK 9: 10: 8	157
1	4	Muriate of potash	1.666	0.8	K	155
2	14	Nitrate of soda Superphosphate Muriate of potash	2.0 3.125 0.833	0.3 0.5 0.4	0.5(NPK) 6: 10: 8	150
1	8	Superphosphate Muriate of potash	6.25 1.666	1.0 0.8	PK 0: 10: 8	148
1	7	Nitrate of soda Superphosphate	4.0 6.25	0.6 1.0	NP 6: 10: 0	124
1	3	Superphosphate	6.25	1.0	P	114
1	2	Nitrate of soda	4.0	0.6	N	109

* The yields are per plot of 1/100 acre each.

† Containing 65.7 per cent. moisture.

‡ Ratio is on a basis of percentage in 1000 lb. per acre of complete fertilizer.

RATIOS OF N, P_2O_5, AND K_2O IN THE FERTILIZERS APPLIED

The fertilizer applied to plot no. 10, tier 1, has been designated the "standard" fertilizer (1). The "standard" fertilizer applied in the pres-

ent experiments at a rate such as to apply 60, 100, and 80 lb. of N, P_2O_5, and K_2O, respectively, per acre is commonly designated as a 6:10:8 fertilizer reflecting the ratios in which the "entities" are present. The amounts and ratios of $N:P_2O_5:K_2O$ used on the other plots are given in table I.

The ratios given in table I for the horse manure are not comparable with the ratios given for the commercial fertilizers. There is no known chemical *in vitro* way of determining the availability of N, P_2O_5, and K_2O in manure. The amounts and ratios given in table I for manure assume complete availability of all the N, P_2O_5, and K_2O present. This is not the case. Very little of the phosphoric acid and nitrogen is soluble in water, although nearly the whole of the potash may be. On the other hand, the amounts of phosphorus and potash fixed by the soil from manure is negligible in comparison with the amounts fixed from the salts present in commercial fertilizers. The availability of the commercial fertilizer added to a soil is contingent upon many properties of the soil (**2, 4, 5, 6**).

Since all the plots examined in this paper have received as dressings all three elements—nitrogen, phosphorus, and potassium—the mode of nutrition of well-nourished plants is being explored. The physiological differences examined, therefore, are those of plants the yields of which are in the higher ranges, whereas in the previous paper (**8**) the physiological differences examined were those of plants the yields of which ranged from poor to good.

Sampling of the leaves

This was carried out in the same manner as previously described. For a complete discussion of method and principles of sampling the earlier paper (**8**) should be consulted, but for convenience a brief outline is presented herewith. Leaves of the same rank (physiological or metabolic age) were taken at each sampling date from all of the plants along a row lengthwise of each plot. Successive rows were then sampled in like manner on each successive sampling date. The leaves chosen from each plant were those immediately above the caducous leaves at the base of a stalk.

In the present experiment the fourth and fifth leaves were taken from two stems issuing directly from the mother tuber of the fourteen plants constituting a row. The samplings were carried out on four dates during July and August, 1935, consecutive rows in each plot being sampled on each successive sampling date.

It has already been pointed out (**8**) that the number of samplings is arbitrary, but at least four should be taken. The number of leaves sampled from each plant also is immaterial but the number should not differ too much from one plant to another. The development of the plants on each plot should be homogenous so that the leaves selected are representative of the plants on the plot.

Presentation of results

The results of the analysis of the leaves constituting the foliar diagnosis are shown in table II expressed as (a) percentages of N, P_2O_5, and K_2O in the dried foliage, (b) milligram equivalents, and (c) the *NPK-unit* (*i.e.*, the N-P_2O_5-K_2O equilibrium).

It is again emphasized that the graph for any treatment is an analytical characteristic of the chemical condition (state) of the selected leaf of plants growing on that plot at the time of sampling. The chemical condition is referred to as the percentage of dry matter in the leaf. Neither the number of leaves sampled from each plant nor the weight of the dry matter are taken into consideration in the method of foliar diagnosis.

Discussion and interpretation of results

NPK-Unit

The *NPK-unit* concept and the method of derivation has been given in an earlier paper (**8**).

The *NPK-unit* represents the equilibrium between nitrogen, phosphoric acid, and potash in the leaf considered at the moment of sampling. The manner of derivation of the *NPK-unit* indicates that it is independent of the values of *s* (column 5, table II), and which represents the total quantity (or *intensity*) of nutrition at the moment of sampling.

Consequently, the *NPK-unit* is a magnitude which expresses the *quality* of nutrition as distinguished from the *quantity* factor (*s*). However, although these two magnitudes are abstractly distinct, they are inseparably connected in the physiological processes of the leaf and must, therefore, be considered simultaneously.

In table II the composition of the *NPK-unit* is represented by the values of X, Y, and Z, shown in the last three columns. For each simultaneous value of X, Y, and Z there is one point and only one point on an equilateral triangle of sides = 100.

Indications given by the graphs

Graphic representation with percentage of elements as ordinate and dates of sampling as abscissa.—Figure 1 shows the graphic representation of the foliar diagnosis of the plants growing on these plots with the percentage of N, P_2O_5, and K_2O as ordinate and the dates of sampling as abscissa.

The facts brought out from an examination of the plants growing on the plots reported in the previous paper (**8**) are true also for the plants growing on the plots examined in the present paper. The graphs (foliar diagnosis) of the differently fertilized plots which have given diverse yields show a regularity which is related to the nature of the different fertilizers in that

TABLE II

COMPOSITION OF LEAVES OF RANK 4 AND 5 AT PERIODIC INTERVALS IN TERMS OF PERCENTAGE OF N, P_2O_5 AND K_2O IN DRIED FOLIAGE, MILLI-

TABLE II

COMPOSITION OF LEAVES OF RANK 4 AND 5 AT PERIODIC INTERVALS IN TERMS OF PERCENTAGE OF N, P_2O_5 AND K_2O IN DRIED FOLIAGE, MILLIGRAM EQUIVALENTS, THE NPK-UNIT, AND THE SUM $(N+P_2O_5+K_2O)$

Date of sampling	Dried foliage				Milligram equivalents				Composition of the NPK-unit		
	N	P_2O_5	K_2O	$M_x+M_y+M_z$	N	P_2O_5	K_2O	(S)	X	Y	Z
	(M_x)	(M_y)	(M_z)	(s)	(E_x)	(E_y)	(E_z)	$E_x+E_y+E_z$	$\left(100\ \frac{E_x}{S}\right)$	$\left(100\ \frac{E_y}{S}\right)$	$\left(100\ \frac{E_z}{S}\right)$
	%	%	%	%	*mg. eq.*	*mg. eq.*	*mg. eq.*	*mg. eq.*	%	%	%
	Manure plot (tier 1, no. 15)										
July 7	4.65	0.688	7.550	12.888	332.010	29.102	160.815	521.927	63.612	5.575	30.812
July 29	3.80	0.608	5.128	9.536	271.320	25.718	109.226	406.264	66.785	6.331	26.885
Aug. 9	3.79	0.564	5.155	9.509	270.606	23.857	109.801	404.264	66.939	5.902	27.161
Aug. 24	3.28	0.448	6.658	9.386	234.192	18.950	120.515	373.657	62.675	5.071	32.254
	N(1.5 P)K plot (tier 2, no. 8)										
July 7	4.84	0.710	6.969	12.519	345.576	30.033	148.440	524.049	65.944	5.730	28.325
July 29	3.72	0.564	4.914	9.198	256.608	23.857	104.668	394.133	67.391	6.054	26.557
Aug. 9	3.46	0.482	4.934	8.876	247.044	20.389	105.094	372.527	66.314	5.473	28.209
Aug. 24	2.86	0.350	4.670	7.880	204.204	14.805	99.471	318.480	64.117	4.650	31.233
	NP(1.5 K) plot (tier 2, no. 12)										
July 7	4.86	0.594	7.798	13.252	347.004	25.126	166.097	538.227	64.470	4.669	30.860
July 29	4.19	0.522	5.767	10.479	299.166	22.081	122.837	444.084	67.368	4.972	27.661
Aug. 9	3.71	0.513	5.782	10.005	264.894	21.699	123.157	409.750	64.647	5.296	30.057
Aug. 24	2.82	0.394	5.996	9.210	201.348	16.666	127.715	345.729	58.239	4.822	36.939
	2(NPK) plot (tier 2, no. 16)										
July 7	5.23	0.682	8.771	14.683	373.422	28.849	186.822	589.093	63.389	4.897	31.713
July 29	4.04	0.562	5.930	10.532	288.456	23.773	126.309	438.538	65.777	5.420	28.802
Aug. 9	3.87	0.500	6.124	10.494	276.318	21.150	130.441	427.909	64.574	4.943	30.483
Aug. 24	3.30	0.354	6.453	10.107	235.620	14.974	137.449	388.043	60.720	3.858	35.422

TABLE II—(*Continued*)

Date of sampling	Dried foliage				Milligram equivalents				Composition of the NPK-unit		
	N	P_2O_5	K_2O	$M_x+M_y+M_z$	N	P_2O_5	K_2O	$E_x+E_y+E_z$	X	Y	Z
	(M_x)	(M_y)	(M_z)	(s)	(E_x)	(E_y)	(E_z)	(S)	$\left(100\ \frac{E_x}{S}\right)$	$\left(100\ \frac{E_y}{S}\right)$	$\left(100\ \frac{E_z}{S}\right)$
	%	%	%	%	*mg. eq.*	*mg. eq.*	*mg. eq.*	*mg. eq.*	%	%	%
	NPK plot (tier 1, no. 10)										
July 7	4.98	0.584	6.589	12.153	355.57	24.70	140.34	520.62	68.30	4.74	26.96
July 29	3.88	0.509	4.337	8.726	277.03	21.53	92.38	390.94	70.86	5.51	23.63
Aug. 9	3.62	0.482	4.697	8.799	258.47	20.39	100.05	376.90	68.22	5.38	26.40
Aug. 24	3.14	0.392	4.348	7.880	224.19	16.58	92.61	333.39	67.25	4.97	27.78
	(1.5 N)PK plot (tier 2, no. 4)										
July 7	5.23	0.614	6.780	12.624	373.422	25.972	144.414	543.808	68.667	4.775	26.555
July 29	4.00	0.488	5.040	9.528	285.600	20.642	107.352	413.594	69.054	4.990	25.956
Aug. 9	3.98	0.464	4.534	8.978	284.172	19.627	96.574	400.373	70.977	4.903	24.120
Aug. 24	3.16	0.356	4.302	7.818	225.624	15.059	91.633	332.316	67.892	4.532	27.572
	0.5 (NPK) plot (tier 2, no. 14)										
July 7	4.84	0.520	6.108	11.468	345.576	21.996	130.100	497.672	69.439	4.418	26.142
July 29	3.77	0.528	4.379	8.677	269.178	22.334	93.273	384.785	69.957	5.803	24.239
Aug. 9	3.34	0.466	4.031	7.837	238.476	19.712	85.860	344.048	69.315	5.729	24.956
Aug. 24	2.84	0.418	3.920	7.178	202.776	17.681	83.496	303.953	66.715	5.817	27.468

(1) the percentages of nitrogen, phosphoric acid, and potash found in the dried foliage reflect the differences in the quantity of the particular element applied in the sense that response to a particular element (as deduced from the development and yield) is shown by a corresponding increase in the percentage of that element in the dried foliage; (2) the graphs for nitrogen, phosphoric acid, and potash, respectively, show by their relative forms and relative positions with respect to the corresponding graphs in the other plots the difference in the nature ("chemism") or course of nutrition with respect to these elements which is correlated with the different fertilizers.

Thus differences in the slopes of two graphs from one sampling date

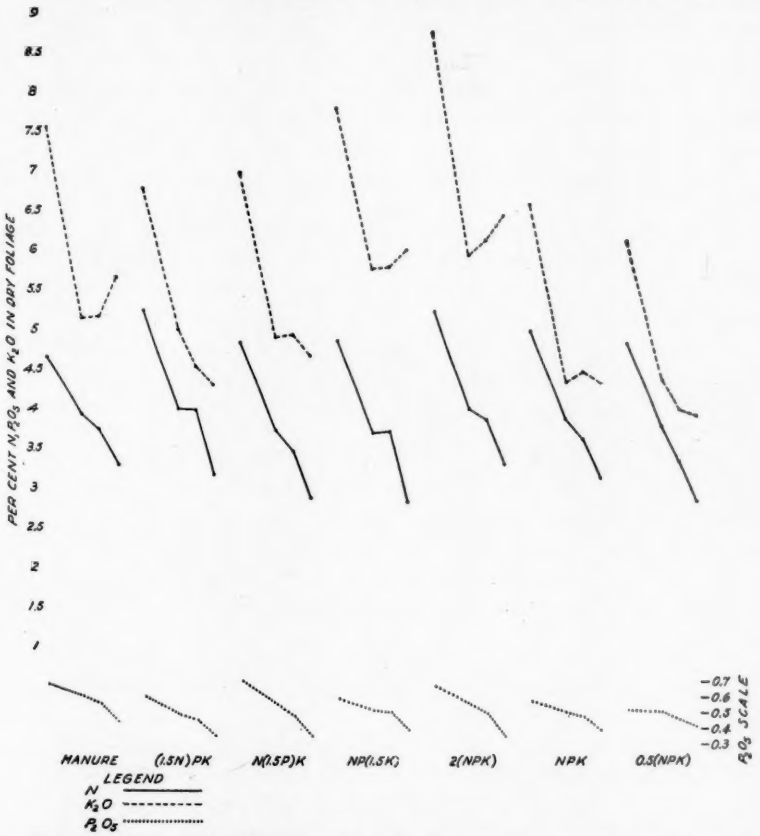

FIG. 1. Values of the dominant elements (expressed in percentage of the dried foliage) at different sampling dates in leaves from plants receiving the different treatments indicated.

to the next indicate differences in the relations of demand for a particular element by the plant to supply of that element to the plant. Hence, if the graph of an element does not sensibly decrease with time, this fact would indicate that the particular element is in relative excess or "bottled up" in the leaf.

Characteristics of the graphs.—(a) *Nitrogen graphs.*—The graphs of the 2(NPK) plot (no. 2–16) and the (1.5 N)PK plot (no. 2–4), both of which received higher nitrogen applications than the other plots, are higher than the other graphs during the first two periods correlated with which is a higher development of the aerial parts. This higher content of nitrogen, however, has not resulted in giving the highest yields of tubers.

The nitrogen graphs of the 0.5(NPK) plot (no. 2–14), the N(1.5 P)K plot (no. 2–8), and the NP(1.5 K) plot (no. 2–12) are similar in position but not always in form. The position of the nitrogen graph of the NPK plot (no. 1–10) is (as we should predict from the facts of the reciprocal effects of the elements on the absorption of nitrogen) intermediate to the graphs of 2(NPK) and (1.5 N)PK on the one hand, and of N(1.5 P)K and NP(1.5 K) on the other.

The graph for the highest yielding plot—the manure plot (no. 1–15)—is unique. The graph for nitrogen is lower than that for any of the above plots at the first sampling date (July 7), but higher than any except that of the 2(NPK) plot at the last sampling date (August 24).

As already stated, the relative steepness (slope) of the graphs is an index of the relative rates of demand and supply of the particular element, a very steep descent indicating a greater demand on the selected leaf relative to supply. The relatively small slope, therefore, of the nitrogen graph of the manure plot (no. 1–15) suggests that the supply, although not at a very high level at the beginning of the sampling (July 7) relative to some of the other plots, continuously keeps pace with the demand by the plant throughout the whole period.

Although 2(NPK) (no. 2–16) contains twice as much nitrogen as that of the "standard" fertilizer NPK, the increase in yield has not been notable and in (1.5 N)PK (no. 2–4) an actual decrease in yield is recorded compared with the "standard" fertilizer. And at the beginning of vegetation the nitrogen contents of 2(NPK) (no. 2–16) and (1.5 N)PK (no. 2–4) are identical.

It would be correct accordingly to conclude that since a content of nitrogen in the leaf in 1935 of 4.86 per cent. was sufficient to give the highest yields obtained, that the content of 5.35 per cent. in 2(NPK) (no. 2–16) is in excess, but that the buffer capacity of the plant has prevented any injury to the yield in 2(NPK) (no. 2–16) but may have been injurious in (1.5 N)PK (no. 2–4) on account of reducing the P_2O_5 content of the leaf.

There is accordingly a relationship between the quantity of nitrogen in the leaf and nitrogen in the fertilizer, but there is no relationship between the quantity of nitrogen in the leaf and the yield nor between nitrogen in the fertilizer and the yield.

(b) *Phosphoric acid graphs.*—The graphs for the N(1.5 P)K plot (no. 2–8), the 2(NPK) plot (no. 2–16) and the manure plot (no. 1–15) are higher than those of any of the other plots during the first two periods, after which greater demand relative to supply causes the graphs of these three plots to fall below those of three other plots. The graphs of the aforementioned plots are similar in form except that the slope of the graphs for the manure plot (no. 1–15) shows the same characteristic as that of the nitrogen graph for this plot, *viz.*, a high level of supply adequate for the demand throughout the whole period.

The graphs for the NPK plot (no. 1–10) and that for the NP(1.5 K) plot (no. 2–12) have nearly the same form and position.

On the other hand, the graph for the (1.5 N)PK plot (no. 2–4) descends below that of the NPK graph (no. 1–10), suggesting that the increment of nitrogen has occasioned a decrease of phosphoric acid in the leaf resulting in a reduction of the yield.

It is significant in this connection to observe that an increment of phosphoric acid in the fertilizer and reflected in the leaf [*cf.* N(1.5 P)K (no. 2–8) with NPK (no. 1–10)] and also an increment in the potash in the fertilizer and also reflected in the leaf [*cf.* NP(1.5 K) (no. 2–12) with NPK (no. 1–10)] have in both cases resulted in increased yields by reducing the nitrogen content of the leaf.

In 1935 a content of phosphoric acid in the dried leaf of 0.68 per cent. in the early stages of vegetation has been sufficient for maximum growth. In N(1.5 P)K (no. 2–8) the content of P_2O_5 has somewhat exceeded this without detriment, however, to the yield.

Accordingly, there is a relationship between phosphoric acid in the leaf and its presence in the fertilizer. There is, however, no relationship between phosphoric acid in the leaf and the yield and none between the quantity of phosphoric acid in the fertilizer and the yield.

(c) *Potash graphs.*—The graphs for the 2(NPK) plot (no. 2–16), the NP(1.5 K) plot (no. 2–12), the manure plot (no. 1–15), and the N(1.5 P)K plot (no. 2–8) are higher than any of the other plots throughout the whole period. It is significant, too, that these four plots are the highest yielding plots in tiers 1 and 2. The graphs for the three first mentioned plots are similar in form but not in position. The relatively very steep slopes of the graphs of the 2(NPK) plot (no. 2–16) and also of the manure plot (no. 1–15) during the first period indicate a greater demand on the potash relative to supply to the plants growing on these plots. Commencing with the

second period, accumulation of potash ("luxuskonsumption") (3) occurs in the leaves of plants growing on these plots.

In the commercial fertilizer plots a content of potash of 7.79 per cent. in the dried foliage at the earlier stages of vegetation has been sufficient for maximum yields. The potash content of the leaves of 2(NPK) has exceeded this limit by about 1 per cent., but has not resulted in a serious reduction in the yield.

The buffer capacity of the plant, therefore, is very great with respect to potash in plants receiving all three elements, nitrogen, phosphorus, and potassium. This is probably one reason for the success of complete fertilizers, although they may not be properly equilibrated.

There is, accordingly, a relationship between potash in the leaf and potash in the fertilizer, between the quantity of potash in the leaf and the yield, and between potash in the fertilizer and the yield.

In the earlier paper (8) it was pointed out that the basic assumption in field plot experiments is that the factors other than those introduced are nearly equal. On this basis the development and yield is related to the composition of the leaf with respect to nitrogen, phosphoric acid, and potash. The equilibrium between these elements will now be examined.

GRAPHIC REPRESENTATION OF THE COURSE OF NUTRITION AS SHOWN BY THE RESPECTIVE COMPOSITE *NPK-UNITS*.—The relationship of the $N\text{-}P_2O_5\text{-}K_2O$ equilibrium to yields of tubers is readily shown by means of graphs in which the *NPK-unit* is plotted at each successive sampling date in trilinear coordinates.

The derivation of the composite *NPK-unit,* which indicates the equilib-

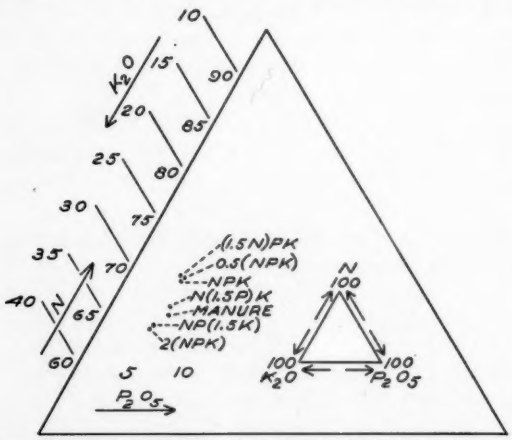

FIG. 2. Mean $N\text{-}P_2O_5\text{-}K_2$ equilibrium (mean *NPK-units*) of leaves from plants receiving the different treatments indicated.

rium between N-P_2O_5-K_2O at the moment of sampling, has been given in an earlier paper (8). The composition of the *NPK-units* at each sampling date for each of the plots is given in the last three columns of table II.

Mean NPK-unit and mean intensity of nutrition.—The relationships to each other of the *NPK-units* of leaves from plants growing on the respective plots can be shown at a glance by means of a point (fig. 2). Any one point is the coordinate of the mean *NPK-units* at the successive sampling dates of the fourth leaf from plants growing on a particular plot. These points are, therefore, the mean values of X, Y, Z given in table II and correspond to the center of gravity (C.G.) of the detailed diagrams given in figures 4, 5, 6, and 7.

The *intensities of nutrition* of the leaves of plants growing on the respective plots are plotted in figure 3. These are the sums of the percentages of

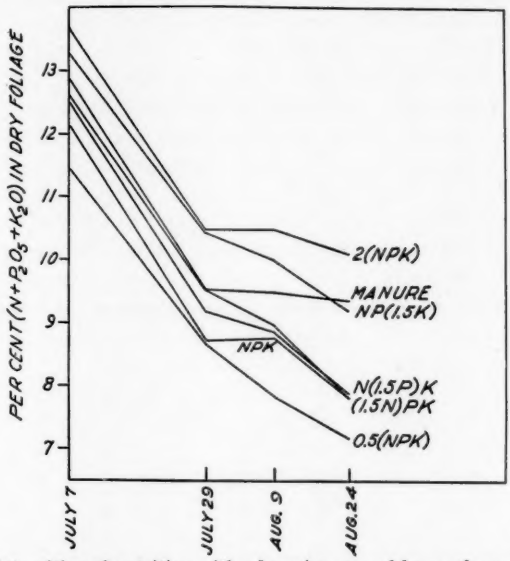

FIG. 3. Intensities of nutrition with advancing age of leaves from plants receiving the different treatments indicated.

($N + P_2O_5 + K_2O$) in the dried foliage at successive dates. The values at any sampling date correspond to the magnitude *s* (column 5, table II).

The mean *NPK-unit* together with the mean *intensities of nutrition* are tabulated in table III together with the absolute and relative yields.

Relation of foliar diagnosis to yields of tubers.—These "complete" fertilizer plots can be divided into three groups according to the position of the point representing the mean *NPK-unit* on the triangle (fig. 2).

TABLE III

RELATION OF MEAN NPK-UNIT AND MEAN INTENSITY OF NUTRITION AT FOUR SAMPLING DATES TO YIELDS OF POTATOES ON VARIOUS PLOTS

TREATMENT	TIER	PLOT	MEAN NPK-UNITS (MEAN OF 4 SAMPLINGS)			INTENSITY OF NUTRITION (MEAN OF 4 SAMPLINGS)	YIELDS			
			N	P_2O_5	K_2O		POUNDS PER PLOT	RELATIVE YIELDS	CLASS ORDER	GROUP
							lb.	%		
Manure	1	15	65.00	5.72	29.28	10.33	196	100.0	1	A
N(1.5 P)K	2	8	65.94	5.47	28.58	9.62	176	89.8	2	
NP(1.5 K)	2	12	63.68	4.94	31.38	10.74	174	88.7	3	B
2(NPK)	2	16	63.61	4.78	31.60	11.19	170	86.7	4	
NPK	1	10	68.65	5.15	26.19	9.39	162	82.7	5	C
(1.5 N)PK	2	4	69.15	4.99	26.05	9.74	157	80.1	6	
0.5(NPK)	2	14	68.85	5.44	25.70	8.79	150	76.5	7	

Group A consisting of the manure plot (no. 1–15) and the N(1.5 P)K plot (no. 2–8) having the highest yields occupying a position on the triangle between groups B and C.

Group B consisting of the NP(1.5 K) plot (no. 2–10) and the 2(NPK) plot (no. 2–16) occupying a position on the triangle below and to the left of the manure and N(1.5 P)K plots. The displacement of this group relative to the manure plot is towards the left base apex of the triangle representing $K_2O = 100$, and further away from the summit of the triangle representing $N = 100$, and from the right base apex representing $P_2O_5 = 100$.

Group C consisting of the NPK plot (no. 1–10), the (1.5 N)PK plot (no. 2–4) and the 0.5(NPK) plot (no. 2–14) occupying a position on the triangle also to the left of the optimum (manure) plot, but higher up in the triangle. The displacement of this group relative to the optimum (manure) plot is towards the summit representing $N = 100$, and farther away from the left base apex representing $K_2O = 100$, and from the right base apex representing $P_2O_5 = 100$.

Course of nutrition as indicated by changes in NPK-unit at each successive sampling.—The displacements, from one sampling date to another, of the *NPK-unit* of the leaves selected from plants growing on the respective plots are given in figures 4, 5, 6, and 7.

Such diagrams show the changes in the equilibrium between nitrogen, phosphoric acid, and potash (NPK). The detailed graphs for all the plots cannot be shown in the same triangle as in figure 3—in which the mean *NPK-units* are shown by a point—because of the intersection of lines representing plots, the *NPK-units* of which are located too near one another.

(a) *General characteristics of the graphs.*—The zigzag nature of these displacements of the *NPK-units* from one sampling date to another are the result of the influence of meteorological factors, and it is of particular importance to note that the influence of these factors on the $N\text{-}P_2O_5\text{-}K_2O$ equilibrium of a plot varies with the treatment it has received.

The changes in the $N\text{-}P_2O_5\text{-}K_2O$ equilibrium during the first period (July 7 to 29) result in increasing the nitrogen and phosphoric acid in the *NPK-unit* at the expense of the potash in every treatment. These changes are not equal quantitatively. They are greatest in manure (no. 1–15), less in NPK (no. 1–10), 2(NPK) (no. 2–16), NP(1.5 K) (no. 2–12), and in 0.5(NPK) (no. 2–14), and insignificant in N(1.5 P)K (no. 2–8) and (1.5 N)PK (no. 2–4).

No generalization is apparent from these results. The phenomena described are concurrent with a marked rise in the mean daily temperature and satisfactory conditions with respect to water supply (8, table III)—conditions favorable to an increase in the biological processes in the soil. This period is accompanied by the greatest development of aerial parts of any period.

During the second period (July 29 to August 9) the changes in the $N\text{-}P_2O_5\text{-}K_2O$ equilibrium are neither alike qualitatively nor quantitatively. In N(1.5 P)K (no. 2–8) and also in 2(NPK) (no. 2–16) and NPK (no. 1–10) decreases in the nitrogen and phosphoric acid in the *NPK-unit* take place; whereas in (1.5 N)PK (no. 2–4) an increase in nitrogen and a decrease in phosphoric acid and potash in the *NPK-unit* occurs, with practically no change in 0.5(NPK) (no. 2–14) and manure (no. 1–15).

Again, no generalization is apparent. The phenomena are concurrent with a drop in mean daily temperature and relatively low precipitation (**8**, table III).

During the last period (August 9 to 24) the changes in the $N\text{-}P_2O_5\text{-}K_2O$ equilibrium are in the direction of a decrease in the nitrogen and phosphoric acid in the *NPK-unit* at the expense of the potash in all treatments. The changes in the *NPK-units* are not quantitatively the same. They are relatively large in manure (no. 1–15), 2(NPK) (no. 2–16), and in NP(1.5 K) (no. 2–12), and medium in the others. The changes are concurrent with relatively dry conditions and an increase in the mean daily temperature (**8**, table III).

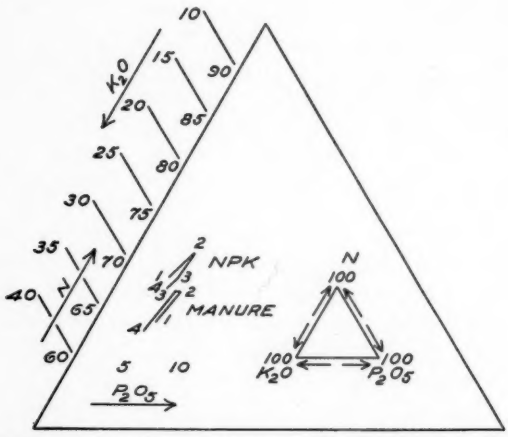

FIG. 4. Course of nutrition as indicated by changes in $N\text{-}P_2O_5\text{-}K_2O$ equilibrium (*NPK-units*) with advancing age of leaves from plants growing on manure and NPK plots. Numerals indicate successive sampling periods.

(b) *Course of nutrition in group A—manure (no. 1–15) compared with N(1.5 P)K (no. 2–8).*—The yield of tubers from the manure plot (no. 1–15) is much higher than from the N(1.5 P)K plot (no. 2–8). The *NPK-unit* of N(1.5 P)K (no. 2–8) is located nearer that of manure (no. 1–15)—the optimum plot—than those of any of the other plots. In fact the *NPK-*

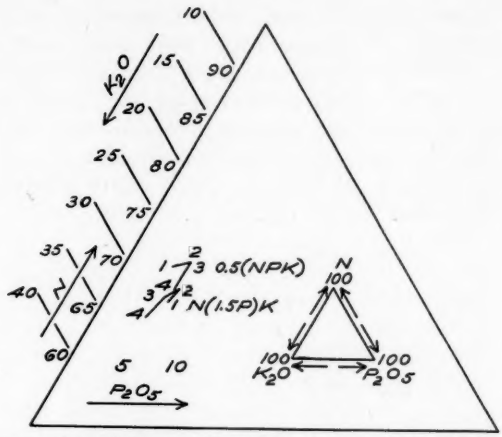

FIG. 5. Course of nutrition as indicated by changes in the N-P_2O_5-K_2O equilibrium of leaves from plants growing on 0.5(NPK) and N(1.5 P)K plots. Numerals indicate successive sampling periods.

units of (no. 1–15) and (no. 2–8) lie so close together that if this factor be considered alone one would expect the yields to be more nearly equal. The fact that the *intensity of nutrition* of this commercial fertilizer plot is much lower than that of the manure plot is significant, and illustrates the necessity of considering both the *quality* and *quantity* of the mineral nutri-

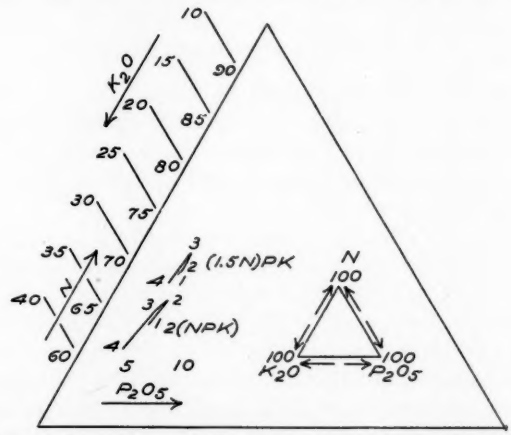

FIG. 6. Course of nutrition as indicated by changes in the N-P_2O_5-K_2O equilibrium of leaves from plants growing on (1.5 N)PK and 2(NPK) plots. Numerals indicate successive sampling periods.

tion. It will be seen from column 3, table II, that this lowered *intensity of nutrition* is principally due to a too low potash content in the case of plants growing on the N(1.5 P)K plot.

Except at the third sampling the graph for N(1.5 P)K (no. 2–8) is displaced higher on the triangle and slightly to the left in relation to the graph of the optimum (manure) treatment. There is accordingly more N, less K_2O, and somewhat less P_2O_5 in the *NPK-unit* of N(1.5 P)K (no. 2–8) than in the *NPK-unit* of manure (no. 1–15).

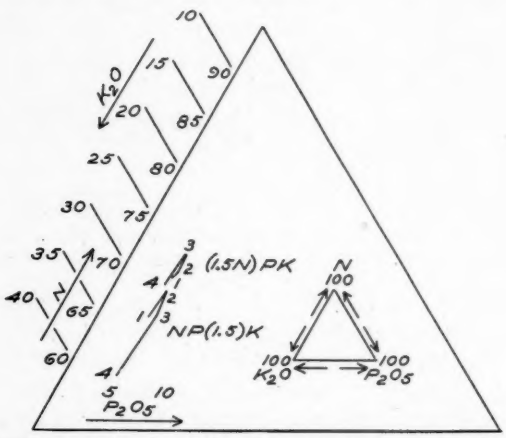

FIG. 7. Course of nutrition as indicated by changes in the N-P_2O_5-K_2O equilibrium (*NPK-units*) with advancing age of leaves from plants growing on (1.5 N)PK and NP(1.5 K) plots. Numerals indicate successive sampling periods.

(c) *Course of nutrition in group B—NP(1.5 K) (no. 2–12) and the 2(NPK) (no. 2–16).*—In comparison with the optimum graph (manure) the *NPK-units* of this group contain with the exception noted less N, less P_2O_5, and more K_2O. (During the first period N in the unit of NP(1.5 K) is greater than in manure (no. 1–15).)

(d) *Course of nutrition in group C—(1.5 N)PK (no. 2–4), NPK (no. 1–10), and 0.5(NPK) (no. 2–14).*—The graphs of (1.5 N)PK (no. 2–4) and of 0.5(NPK) (no. 2–14) are displaced a little higher toward the apex N = 100 per cent. than that of the NPK (no. 1–10). As already indicated the displacements differ quantitatively. Notable is the 30 per cent. increase in the phosphoric acid in 0.5(NPK) (no. 2–14) during the first period.

(e) *Accommodation of the plant to diverse nutritional equilibrium.*—The yields from the N(1.5 P)K plot (no. 2–8) and the NP(1.5 K) plot (no. 2–12) are relatively close together. However, the *intensities* and the *NPK-units* of the two are very different, which may be interpreted to indi-

cate that the cultivated plant is able to accommodate itself to very diverse nutritional equilibriums with but little (if any) effect on the yield, provided that the various equilibriums are accompanied by a sufficient *intensity of nutrition*.

Summary

1. The investigations reported earlier (8) have been extended to other plots of the vegetable fertility experiments of the Pennsylvania Agricultural Experiment Station.

2. The plots examined in this paper were treated with rotted horse manure, and also with complete commercial fertilizers differently equilibrated.

3. The indications given by the two methods of graphic representation (8) are described in detail and the foliar diagnosis of all plots compared with that of the optimum (manure) plot.

4. The *intensity of nutrition* (mean) and the composition of the mean *NPK-unit* (8) of the fourth leaf of potato plants growing in the manure plot is 10.35 and 65.0:5.72:29.3, respectively. This intensity and composition of the *NPK-unit* is tentatively regarded as being near the optimum for the potato (variety Rural Russet) in this year.

The writer desires to express his thanks to Dr. WARREN B. MACK for taking the leaf samples.

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EFFECTS OF A LIMITING ELEMENT ON THE ABSORPTION OF INDIVIDUAL ELEMENTS AND ON THE ANION: CATION BALANCE IN WHEAT[1]

A. G. McCalla and E. K. Woodford

(WITH FIVE FIGURES)

Introduction

Interrelations of the various elements involved in the absorption of minerals by green plants are among the major problems in plant nutrition. Many types of investigation have been carried out and there is available a vast literature on the effect of one ion on another, the differential absorption of ions in single salt solutions, and the accumulation of individual ions. The general effect on absorption of limiting one element has been given some attention, and during the past few years has been under investigation in this laboratory.

The experiments reported in this paper are concerned with the effects of limiting individual elements on the relative absorption of other elements, and on the general $\frac{\text{anion}}{\text{cation}}$ balance in the wheat plant.

There are a number of published results dealing with other phases of nutrition which could be recalculated to yield data of the same type as those discussed in this paper. There are some which have a direct bearing on the present discussion, and while no attempt at a complete review has been made, a brief summary of some of these findings is given.

A comprehensive report of the work of Lundegårdh and his associates is given in a monograph by Lundegårdh (**8**). It is impossible to mention many of the individual results, but interrelations of many important nutrients under conditions of limited supply of one or more of them could be calculated from the data presented. Unfortunately, the nitrogen results are not included, so that the calculation of $\frac{\text{anion}}{\text{cation}}$ ratios is impossible. The interrelations discussed by Lundegårdh are chiefly those among the cations. Increased absorption of calcium, magnesium, and sodium was observed under conditions of limiting potassium supply, but phosphorus (the only anion studied) was not affected. Little or no consistent variation was noted in the ratio of magnesium to calcium. When nitrate was limited there was no

[1] From the Department of Field Crops, University of Alberta, with financial assistance from the National Research Council of Canada. Paper no. 143 of the Associate Committee on Grain Research of the National Research Council and Dominion Department of Agriculture.

apparent effect on phosphorus, but the potassium content of the plants was much reduced.

The question of absorption and interrelations of nutrients has been further discussed by LUNDEGÅRDH (9). Many of the cation interrelations are discussed, and particular attention is drawn to the antagonisms which may exist, and to the effect of one nutrient in modifying the concentration of another within the plant under various conditions of external concentration.

Interrelations of pairs of elements have received considerable attention at the Research Institute of Plant Physiology of the Imperial College (3). The limitation of chemical analyses to the two elements under investigation restricts the comparison of these with our results; but the effect of gradual reduction of one or both elements on the uptake and distribution of the two is of interest. A discussion of the detailed results is out of the question here. It should be mentioned, however, that GREGORY considers that, at low concentrations, the absorption of an element is largely dependent upon its external concentration.

Many examples of interrelations are to be found in the literature. DIKUSSAR (2) reported that when nitrogen was supplied in the form of ammonium there was a depression of the absorption of other cations, the effect being particularly noticeable with potassium. At the same time the absorption of phosphorus was increased. Supplying nitrogen as nitrate increased the absorption of cations and depressed that of phosphorus. The effect of ammonium in depressing the absorption of calcium by tobacco was shown by JACOBSON and SWANBACK (6). It was also demonstrated (14) that when magnesium sulphate was added to a medium previously without magnesium, there was a reduced absorption of potasium and calcium, the latter being forced out into the solution. Many workers have found that phosphate fertilizers increase the phosphorus, and often decrease the nitrogen content of plants. CHAPMAN (1) reported that a nitrogen deficiency caused phosphorus to accumulate in the plant even when the soil was low in phosphorus.

The relations of anions and cations in movement of solutes within the plant are discussed by JACQUES (7). He found a consistently higher equivalent concentration of cations than of anions, but he does not include nitrogen in his calculation of anions.

The uptake of five of the principal elements by lucerne (12) and corn (13) has been studied by RADU. A large number of molecular ratios are presented, but these are all for plants grown under similar conditions. Although the magnitude and trend of some of the ratios are quite different for the two types of plants, there is a marked similarity in the ratio of total anions to total cations. This similarity is also exhibited by results reported by STEPHENSON (15). The mean $\frac{\text{anion}}{\text{cation}}$ ratio for grasses was much the

same as for legumes despite marked differences in nitrogen and calcium content, and consequently in ratios between pairs of individual nutrients.

Material and methods

Five series of wheat plants, all but the first having been grown in water cultures, are considered in this paper. The details of production, collection, and analysis of most of the material have been described elsewhere, so only a brief description of each series is given here. Unless otherwise stated, the plants were grown during the spring and summer. Except for series I, where only the above-ground parts were analyzed, results are for the whole plant.

Series I was grown under field conditions in 1934. The material designated "complete" in the results was grown at Edmonton on fertile black loam soil, and that designated "limiting nitrogen and sulphur" was grown at Fallis, 50 miles west of Edmonton, on relatively infertile gray podsolic loam. Analyses were made on eight individual replicates of each of two varieties of wheat grown on the two soils. Details are given by WOODFORD and McCALLA (**17**). The values given in this paper are means for the two varieties.

Series II was grown in water cultures in 1936, and included "complete" and "limiting nitrogen" ($\frac{1}{2}$ complete) solutions. All solutions were made up in distilled water, and adequate precaution was taken to insure against deficiency of any element other than nitrogen. Limited nitrogen was compensated for by equivalent increases in phosphorus. Analyses were made on duplicate samples at five stages of development. Details are given in another paper (**11**).

Series III was a supplementary series grown in water cultures for 35 days in the fall of 1936. All material received a complete solution for the first six days, after which the six solutions used were as follows: nitrogen-free; limiting nitrogen at $\frac{1}{2}$ and $\frac{1}{4}$ of the complete; complete; limiting phosphorus at $\frac{1}{4}$ of the complete; and phosphorus-free. Limited nitrogen or phosphorus was compensated for by equivalent increases in the other nutrient. There were no other changes in the solutions. At the end of the 35 days the whole crop was harvested and analysed.

Series IV was grown in water cultures in 1934, and included "complete" and "limiting potassium" ($\frac{1}{4}$ of complete) solutions. Although several different conditions of limitation were employed, only that in which limited potassium was compensated for by an equivalent increase in calcium is here considered. Tap water, containing a small amount of sodium, was used in making up these solutions. The first seeding of this series, on which analyses were made at three stages, was unfortunately lost as a result of an accident in the greenhouse when the plants were 44 days old. A second seed-

ing was made, and the plants analyzed at 31 and 54 days of age. The results for the two seedings are recorded separately. Details regarding this series are given in an earlier paper (**10**).

Series V was grown in water cultures in 1936 and included "complete" and "limited calcium" ($\frac{1}{20}$ of complete) solutions. The reduction in calcium was compensated by an equivalent increase in potassium. Duplicate analyses were made at five stages of maturity. The same general procedure was used in production, and the same methods of analyses as for series II.

For all the material grown in water culture, the complete solution used was Hoagland's complete nutrient solution made up as follows: 5 cc. of molar $Ca(NO_3)_2$, 5 cc. of molar KNO_3, 2 cc. of molar $MgSO_4$ and 1 cc. of molar KH_2PO_4 per litre of solution. Ferric tartrate at the rate of 1 cc. of 0.5 per cent. per litre was added every third or second day during the experiment.

Results

Analytical results from these studies are here confined to a minimum. Most of the detailed results have been published elsewhere (**10, 11, 17**), and for the purpose of the present discussion are unnecessary.

In calculating the ratios, two assumptions had to be made. The first was that nitrogen is absorbed as an anion, and, except for the first experiment, this is the only justifiable assumption, since all nitrogen was supplied as nitrate. While this is not proof that it is absorbed as such, it seems certain that nitrate was absorbed in large quantities, because it formed an appreciable proportion of the total nitrogen of the complete culture plants at all stages, and over 50 per cent. of the nitrogen in the leaves and stems at maturity. The field-grown material of series I also contained small amounts of nitrate, and since the various ratios in field- and culture-grown material were much alike, it was assumed the nitrogen was absorbed as nitrate. The second assumption was that phosphorus is absorbed as a divalent anion, an assumption which is possibly not as sound as the first. The reason for this assumption was that in all earlier culture experiments the pH of the solutions very rapidly reached and was maintained at approximately 7.2. At this pH more phosphate is in the form of $HPO_4^=$ than in the form of $H_2PO_4^-$. An accurate allowance for the two forms was impossible, since there were differences in pH from time to time and from culture to culture. If it had been assumed that the absorption of phosphorus took place as the univalent $H_2PO_4^-$ the results and conclusions would have been only slightly affected by this change in procedure. In only one specific instance (series III) was a marked difference in the pH of different solutions found, and this is discussed in its place.

The term "limiting" is used in this paper because the effects of moderate limitation of an element are much more striking than those of a mod-

erate excess. In series II, for example, the solution termed "limiting nitrogen" might have been designated "excess phosphorus." Similarly in the other water culture series, the solutions might have been designated by the element in excess rather than by that in limitation. This is not true of series I because there was no excess phosphorus in the podsol; in fact this element was definitely lower than in the black loam.

All ratios presented in the following sections are on a chemically equivalent basis.

Limiting anions

Series I. Limiting nitrogen and sulphur under field conditions

A summary of the analytical results for wheat grown under conditions of complete (black loam soil), and limiting nitrogen and sulphur (podsolic loam) nutrition is presented in table I. The ratios between individual pairs and between various combinations of nutrients are presented in figure 1. These results are for the above-ground parts only of the plant.

TABLE I

NUTRIENT CONTENT AS PERCENTAGE DRY MATTER OF WHEAT
SERIES I

Nutrient	Soil nutrients	Nutrient content				
		Time from seeding (days)				
		29	48	64	90	115
		%	%	%	%	%
Nitrogen	Complete*	5.62	3.96	2.24	1.40	1.46
"	Limiting N and S†	5.23	1.88	1.08	0.86	0.94
Sulphur (SO_4)	Complete	1.30	1.00	0.73	0.64	0.42
"	Limiting N and S	1.10	0.44	0.31	0.30	0.30
Phosphorus (P_2O_5)	Complete	0.93	0.60	0.37	0.28	0.24
"	Limiting N and S	1.23	0.82	0.52	0.46	0.48
Potassium	Complete	4.38	4.08	2.88	1.16	0.78
"	Limiting N and S	3.69	2.72	1.92	0.97	0.70
Calcium	Complete	0.90	0.60	0.34	0.25	0.20
"	Limiting N and S	0.72	0.35	0.20	0.13	0.16
Magnesium	Complete	0.40	0.23	.....	0.06	0.08
"	Limiting N and S	0.28	0.11	.....	0.06	0.07

* Fertile black loam soil.
† Relatively infertile gray podsolic loam.

The most striking feature of the results is the low nitrogen and sulphur content and the correspondingly higher phosphorus content of the wheat grown on the podsol. All cations were lower in the plants with limited nitrogen and sulphur, but there was no indication that any of these, except possibly calcium to a slight extent, was limiting development. This is more clearly seen from the ratios in figure 1. The limited uptake of nitrogen

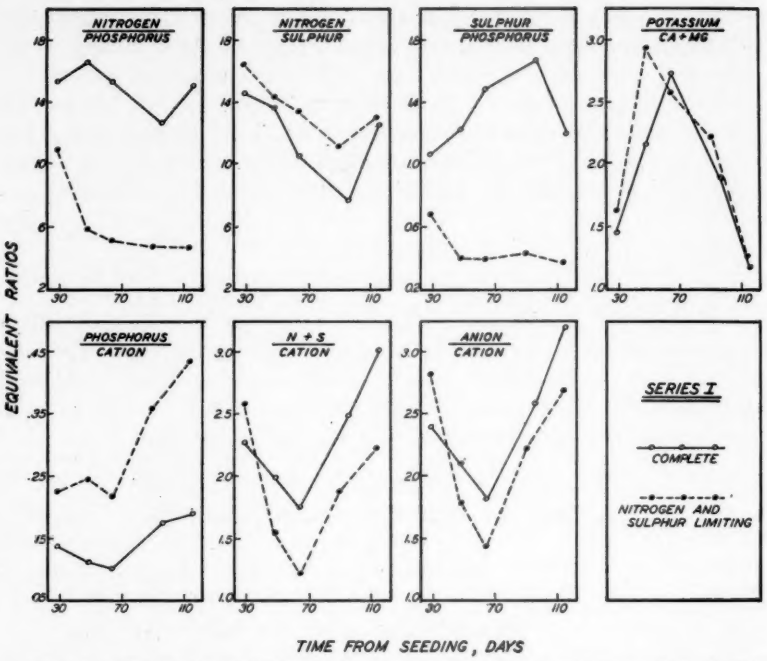

FIG. 1. The effects of limiting nitrogen and sulphur on various equivalent ratios, series I.

and sulphur in relation to phosphorus is clearly evident in these graphs. It is evident also that sulphur was slightly more limiting than nitrogen.

The ratios involving cations show that there was no marked difference in the proportion of potassium, calcium, and magnesium taken up from the two soils. Thus, although all cations were lower in the plants grown on the podsol, this lower level was apparently more closely related to the reduction of total anion absorption than to any relative deficiency in cation supply.

Despite the relatively large differences in nitrogen, sulphur, and phosphorus, the total $\frac{\text{anion}}{\text{cation}}$ balance in the plants grown on the podsol was fairly well maintained both by the increase in phosphorus and the decrease in cation absorption. It must be stressed here that although only percentage results are presented in table I, the ratios are not affected by differences in general level of yield. Therefore, while the total amount of phosphorus absorbed by the plants growing on the podsol was actually lower than that absorbed by the others, the relative absorption was much greater. Similarly, the total weight results (17) show that the actual cation absorption

from the podsol was only about one-fourth that from the black loam, but the relative cation absorption, while less, was not nearly so much less as the weight figures might suggest at first glance. The $\frac{\text{anion}}{\text{cation}}$ balance has been briefly discussed by WOODFORD and McCALLA (17). The general effect of limiting nutrients on this balance is better discussed after the presentation of the results from the other series.

Series II. Limiting nitrogen under culture conditions

A summary of the results for series II is presented in table II, and the various equivalent ratios in figure 2.

TABLE II

NUTRIENT CONTENT AS PERCENTAGE DRY MATTER OF WHEAT
SERIES II

NUTRIENT	SOLUTION	NUTRIENT CONTENT				
		TIME FROM SEEDING (DAYS)				
		29	42	54	66	92
		%	%	%	%	%
Nitrogen	Complete	4.88	3.70	2.39	2.03	1.95
	Limiting N	3.72	2.37	1.57	1.27	1.01
Phosphorus (P_2O_5)	Complete	2.92	2.34	1.83	1.36	1.57
	Limiting N	3.13	2.61	2.09	2.09	2.71
Sulphur (SO_4)	Complete	1.20	1.00	0.73	0.60	0.88
	Limiting N	1.48	1.50	1.23	1.11	1.24
Potassium	Complete	7.20	5.40	3.41	2.77	2.43
	Limiting N	6.53	4.56	2.94	2.43	1.57
Calcium	Complete	0.66	0.52	0.38	0.49	0.45
	Limiting N	0.54	0.40	0.27	0.28	0.30
Magnesium	Complete	0.15	0.23	0.18	0.16	0.20
	Limiting N	0.12	0.12	0.08	0.11	0.14

Before discussing the ratios, attention must be drawn to the much higher phosphorus values for this series than for series I, and the consequent lower $\frac{\text{nitrogen}}{\text{phosphorus}}$ and $\frac{\text{sulphur}}{\text{phosphorus}}$ ratios. The most likely explanation would seem to be that increased phosphorus uptake resulted from the exclusion of other anions when the solutions were made up in distilled water. It has been reported (14) that when chloride is absorbed, the phosphorus content of the plant decreases, and it seems likely that, with the virtual exclusion of anions except nitrate, sulphate, and phosphate from the solution, the increased phosphorus absorption compensated for other anions usually absorbed.

The form and trend of the curves for $\frac{\text{nitrogen}}{\text{phosphorus}}$ ratios are the same as

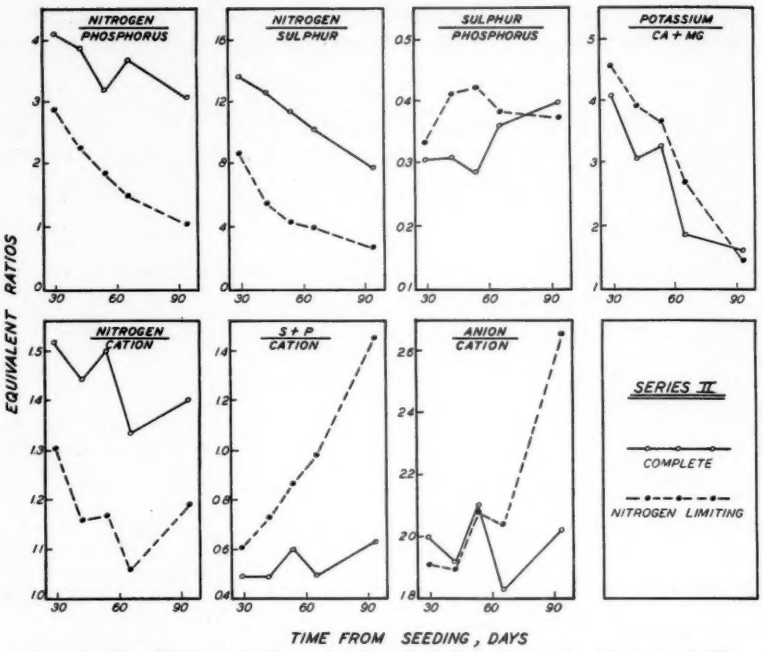

FIG. 2. The effects of limiting nitrogen on various equivalent ratios, series II.

for series I, but the $\frac{\text{nitrogen}}{\text{sulphur}}$ and $\frac{\text{sulphur}}{\text{phosphorus}}$ ratios are, of course, quite different, since sulphur was not limiting in this series. Limiting nitrogen had a negligible effect on the $\frac{\text{sulphur}}{\text{phosphorus}}$ ratio, as the percentage results in table II show that limiting nitrogen increased the relative uptake of both phosphorus and sulphur. There was little effect on the ratios of cation to cation, so that the positions of the curves for the two treatments in the $\frac{\text{nitrogen}}{\text{cation}}$ and $\frac{\text{S}+\text{P}}{\text{cation}}$ graphs are determined by the anion absorption.

The $\frac{\text{anion}}{\text{cation}}$ balance was but slightly affected by the nutrition during the first half of the growth period. The sharp upward swing of the curve for limited nitrogen plants can be discounted considerably, because a large proportion of the marked phosphorus increase (table II) was in the roots only, and there is good reason to believe that probably most of this increase was due to phosphorus deposited on the surfaces of the roots. These roots yielded ash solutions which were brown with iron salts, but most of this

iron had apparently not been absorbed, and was probably deposited on the surface as iron phosphate. Had the importance of this effect been realized at the time of analysis, allowance could probably have been made for it. The fact that the $\frac{\text{anion}}{\text{cation}}$ ratio in the mature limited-nitrogen plants is high, instead of low as in series I, adds evidence in favor of the idea that not all of the phosphorus was absorbed.

Series III. Limiting nitrogen and phosphorus under culture conditions

The results for series II appeared so definite that it was decided to check them by using a graded series of nitrogen and phosphorus concentrations. Owing to the short period of time during which good light conditions were available in the fall, and to the limitation of space for different treatments, these plants were grown only for 35 days. The analytical results are presented in table III and the various ratios in table IV. The compensating

TABLE III

NUTRIENT CONTENT AS PERCENTAGE DRY MATTER OF WHEAT
SERIES III, ALL 35 DAYS FROM SEEDING

NUTRIENT	NUTRIENT CONTENT					
	SOLUTION					
	N-FREE (AFTER 6 DAYS)	LIMITING N ($\frac{1}{40}$ COMPLETE)	LIMITING N ($\frac{1}{20}$ COMPLETE)	COMPLETE	LIMITING P ($\frac{1}{40}$ COMPLETE)	P-FREE (AFTER 6 DAYS)
	%	%	%	%	%	%
Nitrogen	2.50	3.18	3.47	3.91	3.74	3.77
Phosphorus (P_2O_5)	6.61	5.99	4.32	3.38	0.62	0.51
Sulphur (SO_4)	2.40	1.68	2.25	1.56	1.20	1.17
Potassium	5.38	5.24	5.79	5.69	4.87	3.95
Calcium	.....	0.19	0.26	0.54	0.28	0.28
Magnesium	0.30	0.17	0.29	0.33	0.24	0.29

effect of phosphorus under low nitrogen conditions is definitely borne out by these results. Under limiting phosphorus nutrition the reverse effect was not observed, but there was a decided depression (due in large part to potassium) in the amount of total cation absorption.

The $\frac{\text{anion}}{\text{cation}}$ ratios were fairly constant; only under the two most limiting nitrogen conditions were they definitely higher than for the complete. In these solutions, however, the pH values were decidedly lower than that of the complete, the difference being as great as from 5 to 7.2. This difference may have had an effect on the form in which phosphorus was absorbed, and

TABLE IV

EQUIVALENT RATIOS
SERIES III

RATIO	NUTRIENT CONTENT					
	SOLUTION					
	N-FREE (AFTER 6 DAYS)	LIMITING N ($\frac{1}{40}$ COMPLETE)	LIMITING N ($\frac{1}{20}$ COMPLETE)	COMPLETE	LIMITING P ($\frac{1}{40}$ COMPLETE)	P-FREE (AFTER 6 DAYS)
N/P	0.96	1.34	2.04	2.93	15.5	18.4
N/S	3.57	6.47	5.27	8.65	10.8	11.2
S/P	0.27	0.21	0.39	0.34	1.43	1.65
N/cation	1.07	1.44	1.34	1.39	1.68	1.94
S/cation	0.30	0.22	0.25	0.16	0.16	0.17
P/cation	1.12	1.07	0.66	0.47	0.11	0.11
K/Ca + Mg	3.50	5.52	3.96	2.68	3.70	2.65
Anion/cation	2.49	2.73	2.25	2.03	1.95	2.21

a recalculation of the $\frac{\text{anion}}{\text{cation}}$ ratios, assuming phosphorus to be absorbed as $H_2PO_4^-$, makes the levels for the various treatments more nearly uniform, the values for the complete and N-free plants being 1.79 and 1.93.

Both the compensating effect of phosphorus and the generally uniform anion–cation balance are illustrated by the bar diagrams in figure 3. Equivalent percentages, rather than equivalents absorbed, are plotted in

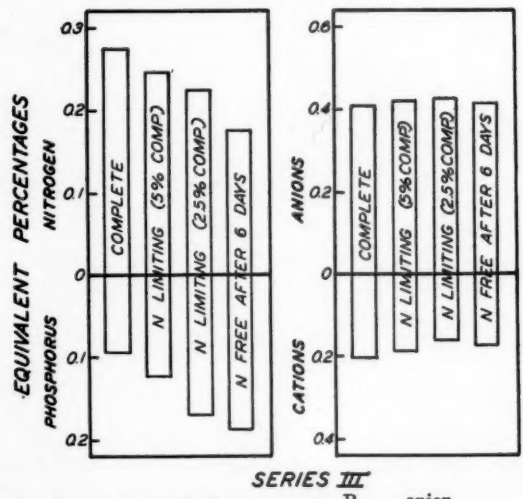

FIG. 3. The effect of limiting nitrogen on the $\frac{P}{N}$ and $\frac{\text{anion}}{\text{cation}}$ ratios, series III.

order to eliminate the differences in level of yield from the various treatments. The relative relationship would be unaltered by plotting weights instead of percentages.

The absorption of nitrogen and phosphorus was roughly parallel to the concentration supplied in the culture solution, especially in the limiting nitrogen cultures. Calculations of the actual milligrams of nitrogen and phosphorus absorbed shows that under no treatment was the absorption of the limited element reduced to a level corresponding with the reduction in concentration supplied; nor was the absorption of the element in excess increased to a level corresponding with the increase in concentration. Furthermore, while the concentration of nitrogen in the low phosphorus solutions was higher than in the complete, the absorption was definitely less. In the plants grown in these solutions the ion balance was maintained by decreased cation absorption, so concentration could have played little part.

Limiting cations

Series IV. Limiting potassium under culture conditions

A summary of the analyses of plants in series IV is presented in table V, and the equivalent ratios in figure 4. These results cover the first 54 days

TABLE V

NUTRIENT CONTENT AS PERCENTAGE DRY MATTER OF WHEAT
SERIES IV

Nutrient	Solution	Nutrient content				
		Time from seeding (days)				
		First seeding			Second seeding	
		20	31	44	31	54
		%	%	%	%	%
Nitrogen	Complete	5.52	5.43	4.98	4.30	3.54
	Limiting K	4.93	4.76	3.92	3.82	2.87
Phosphorus (P_2O_5)	Complete	3.93	2.86	2.39	1.53	1.03
	Limiting K	3.29	2.51	2.38	1.58	1.10
Potassium	Complete	6.38	6.88	7.06	6.13	5.68
	Limiting K	2.14	1.99	1.49	1.80	1.80
Calcium	Complete	0.58	0.72	0.74	0.70	0.57
	Limiting K	0.94	1.14	1.30	1.13	1.14
Magnesium	Complete	0.36	0.32	0.32	0.30	0.30
	Limiting K	0.54	0.49	0.53	0.41	0.46

of development only, and are for two sets of plants as described in the section on material. In general, the percentage results were higher for the 31-day-old plants of the first set than for those of the second, but the ratios

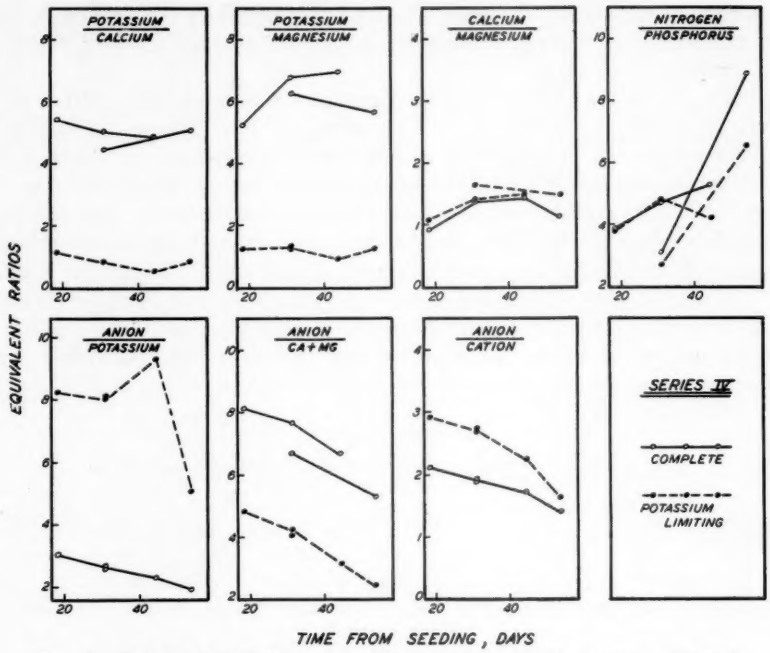

FIG. 4. The effects of limiting potassium on various equivalent ratios, series IV.

are, for the most part, in excellent agreement, and indicate that the error of culture technique was relatively low.

The limitation of potassium resulted in an increased relative absorption of both calcium and magnesium, but the proportions of the two divalent cations was unaffected. These results are in agreement with those already cited (**14**) and with those of HOAGLAND (**4**), who points out that very few cases of interrelations between potassium and magnesium have been reported.

While the $\frac{\text{nitrogen}}{\text{phosphorus}}$ ratios were irregular over the period of the experiment, there was little if any significant difference as a result of potassium limitation. In this series, as in series II and III, the $\frac{\text{nitrogen}}{\text{phosphorus}}$ ratios were much lower than those found under field conditions. While tap water was used in making up these solutions, the only anion whose concentration was appreciably increased by this procedure was sulphate, which was constant for the different solutions.

The relations between anions and potassium, anions and calcium and

magnesium, and anions and total cations again emphasize the tendency for the $\frac{\text{anion}}{\text{cation}}$ balance in plants growing under restriction of one nutrient to be maintained by increased absorption of other ions of the same sign. The difference between the two curves for $\frac{\text{anion}}{\text{cation}}$ ratios might have been much less had sodium been determined. Sodium was present in the tap water, and although it appears that the normal, completely nourished wheat plant absorbs comparatively little sodium (**5, 8**), one limited as to potassium supply may decidedly increase this absorption (**8**).

Series V. Limiting calcium under culture conditions

A summary of the analytical results for series V is presented in table VI, and the equivalent ratios are given in figure 5.

TABLE VI

NUTRIENT CONTENT AS PERCENTAGE DRY MATTER OF WHEAT
SERIES V

NUTRIENT	SOLUTION	NUTRIENT CONTENT				
		TIME FROM SEEDING (DAYS)				
		29	42	54	66	92
		%	%	%	%	%
Nitrogen	Complete	4.88	3.70	2.39	2.03	1.95
	Limiting Ca	4.62	2.98	2.35	2.01	1.78
Phosphorus (P_2O_5)	Complete	2.92	2.34	1.83	1.36	1.57
	Limiting Ca	2.68	2.19	1.61	1.79	1.47
Sulphur (SO_4)	Complete	1.20	1.00	0.73	0.60	0.88
	Limiting Ca	1.14	0.80	0.77	0.74	1.07
Potassium	Complete	7.20	5.40	3.41	2.77	2.43
	Limiting Ca	5.29	4.15	3.30	3.13	2.39
Calcium	Complete	0.66	0.52	0.38	0.49	0.45
	Limiting Ca	0.31	0.27	0.15	0.18	0.17
Magnesium	Complete	0.15	.....	0.18	0.16	0.20
	Limiting Ca	0.18	.....	0.21	0.22	0.38

The limitation of calcium resulted in increased relative absorption of both potassium and magnesium, with the increase in magnesium considerably the greater. Sodium did not enter the cation balance of this series because distilled water was used in all solutions.

The limitation had no effect on the $\frac{\text{nitrogen}}{\text{phosphorus}}$ ratio. The $\frac{\text{anion}}{\text{calcium}}$ ratios were much higher for the plants limited as to calcium than for the complete,

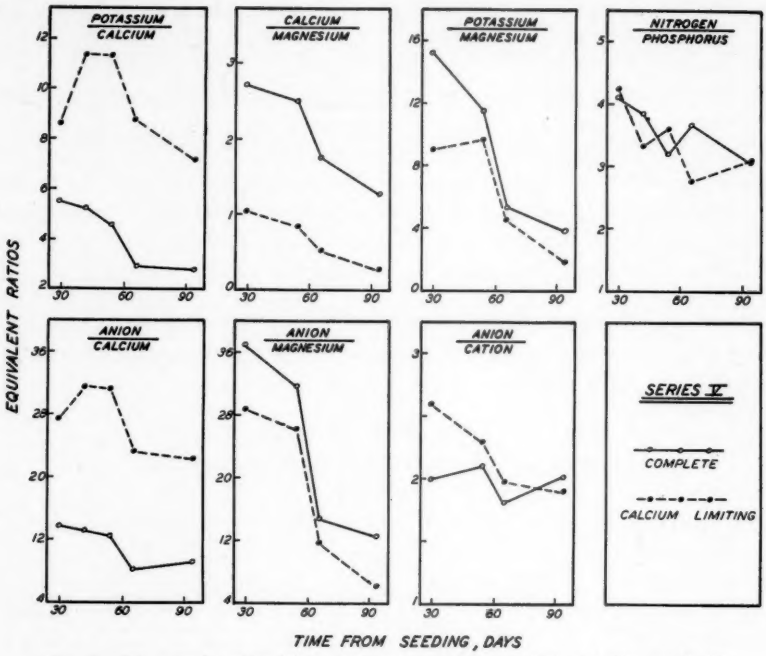

FIG. 5. The effects of limiting calcium on various equivalent ratios, series V.

but the compensating effect of magnesium and potassium reduced the differences in $\frac{\text{anion}}{\text{cation}}$ ratios to a relatively low level. The general effects of limitation were, therefore, of the same nature as in all other series.

Discussion

Most of the material used in this study was produced for purposes other than those discussed, and while the experiments reported in this paper deal with absorption and interrelations of various nutrients, it is recognized that they have little in common with the precise, short-period absorption experiments carried out by STEWARD (16) and others. There is here no possibility of dealing with absorption as distinct from growth. Obviously, most of the nitrogen, a large part of the phosphorus and sulphur, and some part, at least, of the cations determined, are in metabolized form. The results as here reported give the total absorption over definite periods, and as such reflect the interrelations of the various nutrients regardless of the exact mode of absorption, or the external and internal factors affecting this

process. The nature of our experiments makes any application of the results to the mechanism of absorption unwarranted.

Under conditions of growth which were similar, except for the mineral nutrition, there was a consistent tendency toward a maintained balance between anion and cation absorption. In all except the field-grown series I, the plants receiving different treatments were grown together under the same conditions of light, temperature, etc.

The ion balance was always decidedly in favor of the anions, regardless of the nutrition of the plant. This balance is directly the opposite of that discussed by JACQUES (7), and the difference is, of course, accounted for by our inclusion of nitrate in calculating the ratios. In this work the interest is in the uptake of nutrients from the soil, while JACQUES discusses the movement from cell to cell within the plant. While a complete discussion of this question is out of order here, it must be stated that we cannot accept the reasoning of JACQUES, particularly if this is extended to the absorption of nutrients from the soil. Nitrogen cannot be neglected in absorption, nor can absorption from the soil be assumed to take place as equal amounts of NO_3^- and NH_4^+. It is sufficient to say, perhaps, that it is difficult to reconcile the presence of nitrogen-containing organic acids and the coincident movement of a strong hydroxide at the pH's which JACQUES regards as obtaining in the cells under discussion. The argument that nitrogen is present in higher equivalent concentration than the total cations is in agreement with most of our results; but ion exchange, which is casually dismissed by JACQUES, may account for the preponderance of anions over cations. As already mentioned, our results do not permit the drawing of any conclusions regarding mode of absorption, but in any case, the reasoning of JACQUES as applied to absorption by higher plants does not make adequate provision for the uptake of nitrogen.

The other definite result obtained is the effect of a limiting nutrient in increasing absorption of another ion of the same sign or in decreasing the total absorption of ions of the opposite sign. If nutrients were absorbed independently, or if cations had no effect on anions, there seems to be no good reason why the limitation of nitrogen, for example, should be so consistently accompanied by increased absorption of phosphorus or decreased cation absorption. The nearly equivalent compensation by phosphorus of the limited nitrogen uptake in series III indicates that phosphorus absorption was increased to maintain a fairly definite $\frac{\text{anion}}{\text{cation}}$ balance. In the limiting phosphorus cultures of series III the balance was maintained by a decrease in cation absorption, while in series I both of the factors were operative.

Another possible explanation of some of our results, particularly those

of the limited nitrogen cultures in series III, is that the absorption of an element by the plant depends on the external concentration of the element. Thus high phosphorus absorption by plants grown in limited nitrogen solutions would be the result of increased concentration of phosphorus rather than of decreased absorption of nitrogen. Certainly where concentrations are low, there is a direct relation between absorption over short periods and external concentration (3). This explanation of the results cannot be entirely dismissed, since in many of our experiments it is impossible to separate the two effects, but in many other instances of apparent compensation, concentration effects must have been very secondary. A brief consideration of a few such cases may be in order.

The higher phosphorus absorption by the plants grown on the podsol (series I) was in no way related to concentration, because both total and available phosphorus were lower in the podsol than in the fertile black loam from which the plants removed relatively much less phosphorus.

The fact that more phosphorus was absorbed from solutions made up in distilled water than from soil, could scarcely be a concentration effect since the $\frac{\text{nitrogen}}{\text{phosphorus}}$ ratio in the solutions was much higher than in the soil. Similarly the increased phosphorus absorption by plants as a result of nitrogen limitation in series II was not proportionately greater than the increase in sulphur absorption, despite the fact that the external phosphorus concentration had been increased to more than eight times that of the complete solution, while the sulphur concentration was unchanged.

The absorption of calcium and magnesium was increased by limitation of potassium in series IV, the increases being proportionately the same, although only calcium concentration was increased in the solution. Magnesium absorption was increased more than that of potassium when calcium was limited in series V, although only potassium concentration was increased.

In all of these experiments, the concentration of any element in the complete solution was probably sufficient to give maximum absorption, and therefore increases in absorption as a result of increased concentration in the solution would not necessarily be expected. The increased absorption under these conditions, the fact that the increase was not confined to the element in excess, and the fairly constant $\frac{\text{anion}}{\text{cation}}$ balance are all evidence in favor of the compensation rather than the concentration effect.

In all series involving a limitation of nitrogen there appears to be an accompanying decrease in calcium absorption. In series IV, however, the relatively enormous increase in calcium absorption is accompanied by a somewhat decreased nitrogen uptake. In series V, the limitation of cal-

cium had little effect on nitrogen uptake, so the apparent relationship between the two nutrients, as indicated by series I to III, seems open to question. In series I and II, in which nitrogen uptake was limited, the decrease in calcium was only slightly greater than that in potassium absorption; while in series III the uptake of calcium was retarded by limitation of both nitrogen and phosphorus. Apparently only by studying the effect of the two elements at the same time can reliable conclusions regarding interrelations be obtained, and some such system as that used by GREGORY and his students (3) appears to offer the best means of studying specific pairs of elements. Such systems would yield excellent material for studying the effect of limiting nutrients on the total $\frac{\text{anion}}{\text{cation}}$ balance providing analyses were extended to include all of the principal elements involved in absorption. Such studies are now being carried out in this laboratory. Because all the apparent interrelations between pairs of elements not definitely under investigation in this work are subject to the same limitation as those between nitrogen and calcium, no others are considered.

Summary

1. Limiting the supply of one nutrient resulted in an increased uptake of another nutrient absorbed as an ion of the same sign, or in a decreased total uptake of the nutrients absorbed as ions of the opposite sign.

2. When nitrogen was limiting, the effect was chiefly felt in increased phosphorus absorption, although there was also increased sulphur absorption in some series. In one particular series of graded nitrogen and phosphorus supply, the increased phosphorus absorption resulting from limiting nitrogen supply was fully equivalent to the decreased nitrogen uptake.

3. Limiting potassium was accompanied by increased absorption of both calcium and magnesium. Limiting calcium was accompanied by relatively increased absorption of potassium and magnesium and slightly decreased absorption of anions.

4. Despite relatively enormous differences in ratios of individual nutrients as a result of limiting one, there was a marked tendency toward a maintained balance between total anions and cations. This balance was always in favor of the anions.

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RELATION BETWEEN LIGHT AND THE ELECTRIC POLARITY OF *CHARA*

SIDNEY O. BROWN

(WITH SEVEN FIGURES)

Introduction

The purpose of this investigation was to study the nature of the effect of light on the electric polarity of *Chara* and to determine the possible relation of the electric polarity to photosynthesis. As a basis for the final experiments, the normal distribution, magnitude, the degree of stability of the electrical potentials, and the effect of mechanical stimulation were first determined.

An electrical polarity corresponding to the morphological polarity in the axis has been established and studied in detail in *Obelia* and other hydroids (**2, 9**), the Douglas fir (**14**), the onion root, (**11, 19**), frog skin (**3, 15**), *Avena* coleoptile (**5,** WILKS, in press), *Phaseolus vulgaris* (**27**) and other polar structures. These continuously maintained E. M. F.'s have been shown to be quantitatively linked with the oxidation mechanisms of the living cell (**12, 25, 26**). The E. M. F. appears to depend upon the flux equilibrium in an electrochemical system made up of substances necessary for cell oxidation, and in the presence of atmospheric oxygen (**10**). The polarities of these living systems have been shown to be affected by changes in temperature, anaesthetics, oxygen, mechanical stimulation, light, externally applied E. M. F.'s, and other factors, which, directly or indirectly, affect cell oxidation. From the numerous facts obtained from this work LUND (**12**) has formulated a theory of cell correlation which attempts to explain the method by which a group of electrically dominant cells may control the rate and orientation of physicochemical processes in adjacent cells. Some of the new facts presented in this paper appear to have a bearing on this problem.

Previous investigations on the electrophysiology of *Chara* are those by JOST (**8**), who determined the effect of various electrolytes on the potential, and by AUGER (**1**), who reported that stimulation resulted in a negative wave of excitation which moved along the axis at the rate of 6 cm. per second. OSTERHOUT and his co-workers (**22, 23, 26**) have made extensive investigations on the conditions of stimulation and the transmission of the excitatory wave in *Nitella,* a close relative of *Chara.* As far as the writer is aware, the effect of light on the electrical potential of neither *Chara* nor *Nitella* has ever been investigated.

Materials and methods

The material used in this series of investigations was grown in aquaria in a greenhouse. About 4 cm. of the apices of plants from the pond were planted upright in a layer of quartz sand in an aquarium filled with tap water. Light was admitted only from above in order to insure long straight internodes. No fruiting plants were used, because reproductive structures did not appear during the fall and winter months.

The experimental material designated as the "standard preparation" is the apex and the first three normal, straight, vegetative internodes of *Chara*

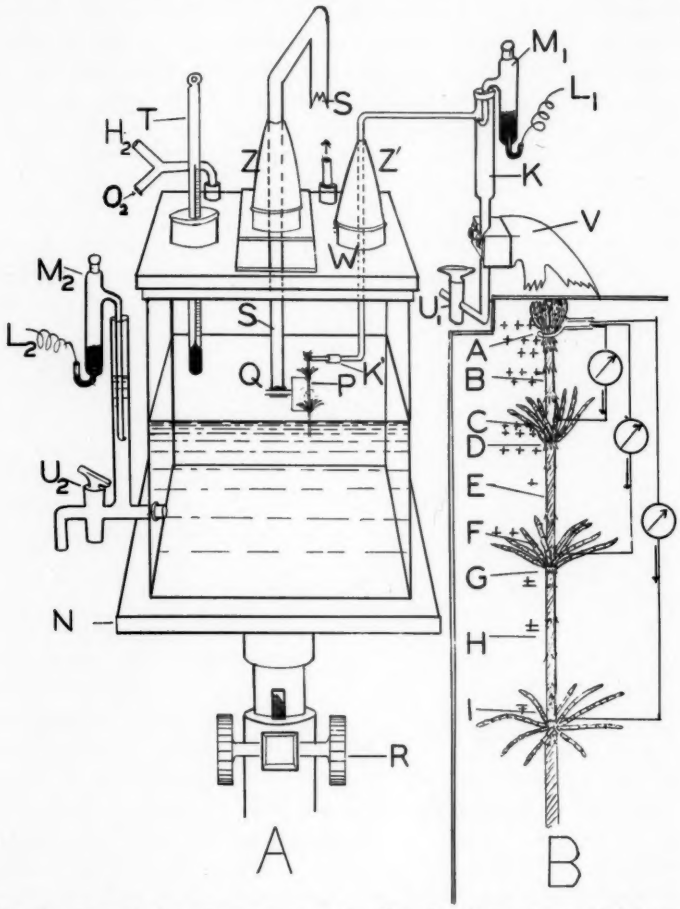

FIG. 1. General view of apparatus used. Full description is provided in the text.

vulgaris. The internodes consist of long multinucleate cells covered by a cortical layer of much smaller spirally arranged cells. At the node, 7 to 10 leaves form a whorl; each leaf has, in general, the same structure as the axis. In figure 1 B the region designated as the apex consists of three or four of the most apical unelongated internodes, covered by leaves of the lower whorls. For experimental purposes the "apex" is, therefore, defined here as all apical structures above the first internode, which is at least 10 millimeters in length. The total axial length of the apex is not more than three millimeters. In figure 1 B the electrode positions along the main axis of the plant are designated by letter. The average length of the first internode was 11 mm., of the second 17 mm., and of the third 20 mm. The part of the "standard preparation" used in the investigation of the effect of light and mechanical stimulation, unless otherwise specified, consisted of the apex, the first internode and node, or that part of the plant between A and D in figure 1 B. If microscopic examination after each experiment showed injury, or if the plant failed to grow on its return to the aquarium, the data obtained from such material were discarded.

An unsuccessful preliminary attempt to determine the electric polarity of the axis of *Chara* indicated that an apparatus had to be constructed which would enable measurements to be made in an atmosphere saturated with water vapor under relatively constant condition of light and temperature with a minimum of mechanical disturbance. Figure 1 A shows the apparatus which was finally constructed and which fulfills these requirements. It consists of a rack and pinion, R, on which is mounted the base, N, supporting a glass aquarium with the inside dimensions of $16 \times 10 \times 8$ cm. A bakelite cover with a large window, W, two small and three large perforations is sealed with a vapor-tight joint to the top of the aquarium. Opaque fingerstalls, Z, form flexible diaphragms over the large openings, to prevent the loss of moisture.

One end of a bent glass rod, S, is attached to a micromanipulator (not shown in the figure). The other end of the glass rod passes through the middle opening and forms a support to which is cemented a tube serving as a receptacle, Q, for a plant holder, H. This receptacle receives a small glass rod cemented to two glass hooks. One of these hooks fits below the first node and the other below the second and serves as a support for the preparation.

The electrode contacts are made (1) by the meniscus at the surface of the water in the aquarium and (2) by a tube, K′, filled with water which connects to the usual small claw contact just below the apex of the plant. The water used in the electrode contacts and in the aquarium is the medium in which the plant was growing. Electrodes M_1 and M_2 of isoelectric zinc amalgam, saturated zinc sulphate type, are connected by leads, L_1 and L_2, to a previously calibrated Compton electrometer. To prevent injury to the

plant by the diffusion of zinc sulphate from the electrodes the contacts were back washed without mechanical disturbance to the plant before each reading by allowing some water to run out through the stopcocks, U_1 and U_2, shown in the figure. The water vapor saturated atmosphere surrounding the plant may be replaced by gases through the openings, H_2 and O_2. During the course of an experiment the temperature did not vary more than 1° C.

Illumination during the determination of the normal orientation and the effect of mechanical stimulation was supplied by means of a 240-candle power frosted bulb, 1 meter above the aquarium. The light was allowed to fall directly on the plant through the glass window, W, in the bakelite cover of the aquarium. In the experiments on light two kinds of illumination were used: (1) illumination of the whole plant with equal intensities from all sides; and (2) illumination of a specific limited region of the plant axis. In obtaining illumination of the whole plant a Mazda type incandescent lamp of 240-candle power, placed at the distance of one meter above the aquarium, was used. The rays were reflected horizontally on the plant by four mirrors, one of which was placed on each side of the aquarium, tilted at 45° angles. Spot illumination was furnished by a galvanometer lamp and slit which focused the light to a band 1.5 mm. in width. A water filter 1.5 cm. thick between the plant and the lamp served to remove heat rays. The intensity of the light was regulated by an iris diaphragm in front of the lamp lens.

Results

Normal electric polarity of the central axis

In order to establish experimental reference conditions, an investigation of the normal inherent polarity was first made. The plant was transferred from the aquarium, in which it had been grown, to the plant holder. All operations were carried out under water with minimum mechanical stimulation. At no time did the plant come into contact with the dry atmosphere. The electrical potential between the apex, A, and the points noted by the letters on figure 1 B was obtained by lowering the water level of the aquarium by means of the rack and pinion, R. With such precautions in manipulation, the apex in the standard preparation was found to be electropositive to the more basal regions. The regions of the greatest change in E. M. F. per unit length are in the first 5 mm. of the plant axis below the apex and across the first node, from C to D (fig. 1 B). In the second internode the electric polarity may at times be completely inverted, the apex negative to the base. In the third internode there is little difference in potential. In determinations made over a period of nine months the apex was positive to the more basal segments in 71 out of 78 plants tested. That is, in 91 per cent. of the cases tested, there was a positive potential gradient from apex to base.

The electric polarity of each plant was determined at intervals of two hours or more for 48 hours or longer and it was found that in the unstimulated condition the apex remained positive. There were a few exceptions to this rule as the apex became negative without obvious external stimulation during the first 24 hours. This was a temporary condition since in 6 to 12 hours the apex again became positive and remained so to the end of the experiment.

In *Chara* the maintained electrical polarity is in agreement with that of other organisms in that the apex, the region of greatest cell activity, is electropositive in the external circuit to the more basal regions.

Effect of mechanical stimulation on the electric polarity

The purpose of the following experiments was to determine the effect of mechanical stimulation on the electrical polarity of the central axis. Two types of stimulation were impressed, first, continuous stimulation produced by a solid object, the glass contact, constantly touching the apex of the plant over an extended period of time; and, second, intense stimulation produced by the tap of an electromagnetic stimulator, which resulted in temporary mechanical deformation similar to that used by Marsh (**20**).

A slow inversion of the electrical polarity of the plant occurred when contact between the glass claw of the electrode and the apex of the plant was constantly maintained under water for an extended period of time. The electrical polarity was determined at intervals of two hours by the method previously described. It was found that this slight supporting pressure on the apex of the plant caused a complete inversion of the potential in about four hours. The electrical polarity returned to normal with the apex electropositive in about the same length of time when the glass claw contact was removed. Application of the contact a second time again caused the polarity of the plant to invert.

This experiment was repeated on 12 different plants, involving 29 cases of stimulation, and in all cases the polarity was decreased or inverted. The susceptibility of the electric polarity to slight mechanical stimulation demonstrated the delicate nature of the polarity potential and explains in part the inconsistent results obtained before perfecting the manipulative technique.

Diminution or inversion of the electric polarity by slight mechanical pressure might suggest an explanation of the various thigmotropic responses since much evidence has been obtained that change in morphological polarity of an organism is associated with change in electric polarity.

The device used for the determination of the effect of slight mechanical deformation applied for a short duration of time was a modification of the apparatus previously mentioned (20). It consisted of a signal magnet to

which was cemented a bent glass rod with one side ground to form a smooth flat surface. To the flexible spring another small bent rod was cemented forming, in effect, a small electromagnetic glass hammer which could deliver a sharp tap to any previously selected location on the plant axis. The stimulator was inserted in the aquarium through an opening covered by a rubber diaphragm. All adjustments were made by a micromanipulator. A roughly quantitative control of the strength of the stimulus was obtained by approximately varying the current which passed through the coils of the electromagnet. The time of application of the stimulus was one second. If any of the plants failed to grow after testing, or if microscopic examination revealed injury, the data from such experiments were discarded.

A mechanical stimulus at any point caused the stimulated region to become negative to the unstimulated region. The negative phase produced by this type of stimulation was of short duration, between 1 and 2 minutes. In a typical case the first two stimuli, applied at the apex 4 minutes apart, each caused a decrease of 15 millivolts in the potential between AD. The third stimulus caused a decrease of 14 millivolts and the fourth 13 millivolts. As a result of 12 successive stimuli of the same intensity, applied at intervals of 3 minutes, a negative response was obtained after each stimulus, and the potential change decreased from 15 to 3 millivolts, indicating "adaptation."

With the upper contacts at the apex and the lower at the base of the internode, stimulation at the apex decreases the polarity of the segment, and stimulation at the midpoint between the apex and base produces little or no change. Repetition of stimuli at corresponding points along the second node gave results similar to those of the first node but relatively smaller in magnitude.

In a typical case with the apical electrode at the midpoint (B, fig. 1, B) and the basal electrode at the base of the first internode (C, fig. 1, B), a decrease of 11.5 mv. in the polarity of the internodes was obtained when the stimulus was applied at the apex (A, fig. 1). There was no vibration of the plant in the water contact sufficient to produce this change. When the apical contact was replaced at the apex, and the plant stimulated at the apex, a decrease of 25 mv. was obtained. Essentially the same results were obtained on the second internode. This demonstrates that mechanical stimulation at the apex caused a temporary negative condition in the polarity of a segment 7 mm. from the point of application; therefore a wave of negativity was produced which traversed the distance of 7 mm. No evidence was obtained to indicate that the negative variation traversed the node. Because of the inadequacy of the electrometer to register rapid changes in potential, the phenomena of conduction will not be considered in the present paper.

Effect of light on electric polarity

When the living plant is illuminated, there is a change in the rate of output of electric energy by the plant. This fact was first observed by Haake (**7**) who found that the illuminated region became electropositive to the unilluminated portion and that no change occurred in etiolated petals and leaves under illumination. A. D. Waller (**29**) suggested that a relation existed between photosynthesis and the electrical changes due to illumination. Bose (**4**), using two halves of a banana leaf connected by a salt bridge, obtained changes in electrical potential when one of the leaves was illuminated.

J. C. Waller (**30–31**) observed current changes between electrodes placed at illuminated and unilluminated symmetrical positions on the surface of leaves. According to him, the magnitude of the electrical response upon illumination is dependent on the species and age of the plant used. He stated that the "photoelectric" response in green leaves is dependent upon the photosynthetic process; however, he stated that, even in the absence of chlorophyll, current changes may be obtained upon illumination. Furthermore he attributed the changes in "current" incident to illumination to a lack of equilibrium between oxidation and reduction of a hypothetical "acid" in photosynthesis. He assumed that the positive phase of the current was due to the "reduction of the acid and that the negative phase was due to the oxidation of the acid." Unfortunately he used, as a measuring instrument, a galvanometer which does not allow a clear distinction to be made between a change in E. M. F. and a change in the resistance of the tissue between the electrodes.

Sheard (**27**), working with the sunflower and the *Poinsettia* obtained potential changes as high as 0.3 volt on exposure of the leaves to the ultra-violet and infra-red light. He obtained no effect with illumination by light in the visible spectrum. Glass (**6**) found that local illumination of the apex of the *Elodea* leaves by white light produced an increase in the potential of the apex with respect to the non-illuminated base.

The object of the following experiments is (1) to determine the effect of illumination on the electric polarity of *Chara;* (2) to establish the relation between the response upon illumination and the photosynthetic process; and (3) to relate this response in part, if not totally, to the increase in the oxygen available for intracellular oxidation.

In order to demonstrate that an electrical response produced by illumination is not due to spontaneous inherent changes in the polarity of the preparation, the first step was to determine the magnitude of inherent change, if any, in the polarity of the standard preparation in darkness, over an extended period of time under constant external conditions. The next procedure was to subject the whole plant to light, other conditions remaining the

same, and to observe the change in polarity produced. Since the final object was to determine the effect of continuous and discontinuous illumination of the apex, the interpretation of the results was simplified by observing the effects produced by illumination of one locus of the plant at any given instant.

In order to attempt to establish a relation between photosynthesis and the electrical response to light, the factors known to affect the photosynthetic process were varied and the results of these variations on the electrical potentials were observed. The variable factors were: (1) the intensity of illumination, (2) the wave length, (3) the concentration of carbon dioxide, and (4) the concentration of O_2 in the atmosphere surrounding the plant. Discussion of the well known effects of variations of these factors on the photosynthetic activity is given by SPOEHR (**28**).

If the change of potential in the plant upon illumination is due to an increase of oxygen available for cell oxidation, the electrical response in light or in darkness should be characteristically modified by a change in oxygen tension around the plant. Therefore, to determine comparatively the effect of illumination on the plant in oxygen and in hydrogen (the absence of oxygen) should also be significant.

The plants prior to the experiment were allowed to remain submerged for two hours in the experimental aquarium in darkness so that any effect due to previous mechanical stimulation or a change of light intensity might have diminished or disappeared. Neither the time of the day nor the condition of illumination prior to experimentation could be shown to have any effect on the response of the plant to light. The periods when the plants were illuminated and unilluminated are indicated by the contrasting heavy lines at the bottom of the graphs.

PROCEDURE I. THE ELECTRICAL RESPONSE OF THE PLANT TO LIGHT.

1. *Normal fluctuation of the electrical potential of the standard preparation in air and darkness.*—The basal line of reference, which was assumed to be the normal E. M. F. residing in the unstimulated preparation in darkness and in vapor-saturated atmosphere, was determined. Figure 2 A shows the range of variability of two different typical plants under the above specified conditions during a period of 50 minutes. In neither of these preparations did any large variations of potential take place spontaneously. Records of 10 different plants over periods from 30 minutes to 2 hours show no greater change than 2 mv. per minute or a total of 10 mv. in 10 minutes. In most preparations a slow relatively small steady decrease in polarity occurred as shown by the broken curve in figure 2 A. Because of the previous results, which show that the presence of the contact at the apex of a plant causes an inversion of polarity in several hours, a decrease in the potential would

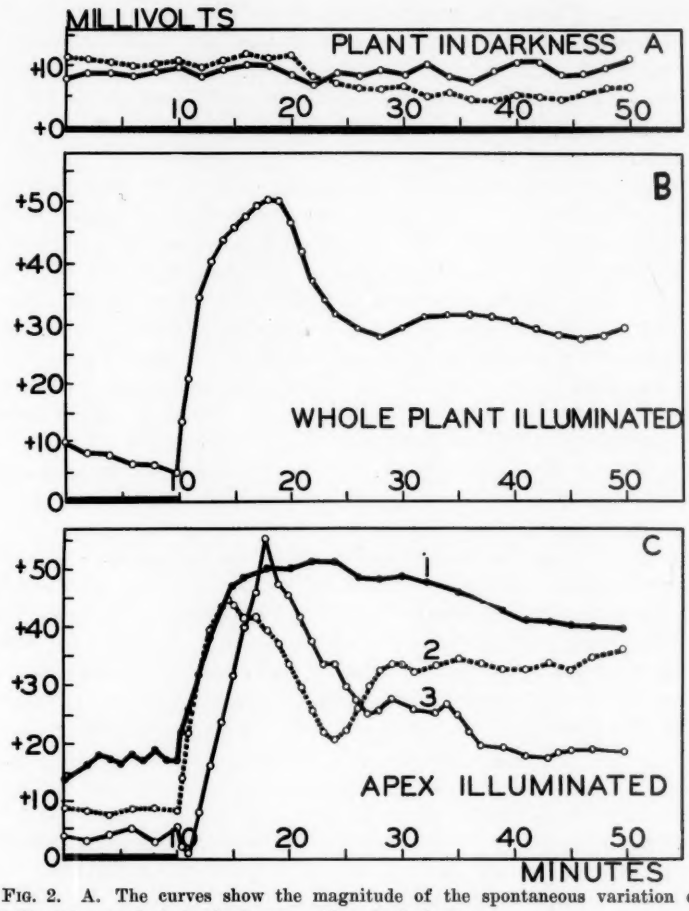

FIG. 2. A. The curves show the magnitude of the spontaneous variation of the E. M. F. of two typical standard preparations in darkness.

B. Effect of continuous illumination of the whole plant on the E. M. F. of the first internode. The light from a 240 c.p. incandescent lamp one meter above the aquarium was reflected on the plant by four mirrors placed on the four sides of the aquarium. The period of darkness before illumination is indicated by the heavy line at the base of the drawing.

C. Effect of continuous illumination of the apex only with light of 36,500 meter-candles on the E. M. F. of the first internode. Curves 1, 2, and 3 represent the responses of three plants under the same conditions.

be expected. *These results may be considered to represent the condition of the maintainance of flux equilibrium concentrations of the electromotively active constituents at a relatively constant value.* Either these curves or the readings taken in all experiments just before application of the light may serve as a control. In no cases were the inherent variations of E. M. F. of the same magnitude as those produced by illumination.

2. *Change in polarity caused by illumination of the whole plant.*—A typical example of the effect of illumination of the entire plant by light of the same intensity is shown in figure 2 B. For this purpose light from a 240-candle power Mazda bulb reflected by a mirror on the four sides of the plant was used. The curve shows a sharp rise in potential to a maximum of 51 mv. The potential sharply decreases to a lower relatively constant value. When the experiment was repeated on several different plants the same type of curves was obtained. In all cases the subsequently maintained polarity was greater than in darkness by at least 50 per cent. *The increase in potential upon uniform illumination affects unequally the potential of the apical and basal regions of the first internode.* This result has a fundamental significance and should be compared to the corresponding effects of oxygen, temperature, and anaesthetics on the electrical polarity in other forms.

3. *Increase of potential upon illumination of the apex only.*—In order to localize the region of greatest change in potential a series of experiments was carried out in which the apex only was illuminated with a spot of light 2 mm. in diameter immediately above the contact. In order to establish a reference level of polarity the potential was observed in darkness for 30 minutes before illumination. Upon illumination, there was an abrupt increase in potential followed by an irregular decline to a relatively constant level. The variability of this response is shown by curves 1, 2, and 3 of figure 2 B. Curve 1, which is typical of most cases, demonstrates an abrupt increase in potential upon illumination, followed by a slow decrease until a relatively high potential level is reached and maintained. In the plant in curve 2, the potential decreases rapidly after the initial difference. Curve 3, characteristic of some cases, illustrates an abrupt increase to a maximum followed by an irregular decrease to a lower constantly maintained polarity. The small initial decrease followed by a steep rise upon illumination, as shown in curve 3, has been obtained in many experiments.

With the claw contact at the apex and the water meniscus just below the first node and with the plant in darkness constant polarity potential of plus 10.0 mv. was obtained. The plant was returned to water and the apex illuminated 10 minutes under water. At the end of this time, with the light still on, the plant was again tested and the potential of the same segment had increased to plus 42 mv. Therefore the increase in potential on illumination is not a phenomenon which takes place only when the plant is out of water and in air.

4. *Electrical polarity of the plant during alternate exposures of the apex to light and darkness.*—Figure 3 A shows the changes which occur in the

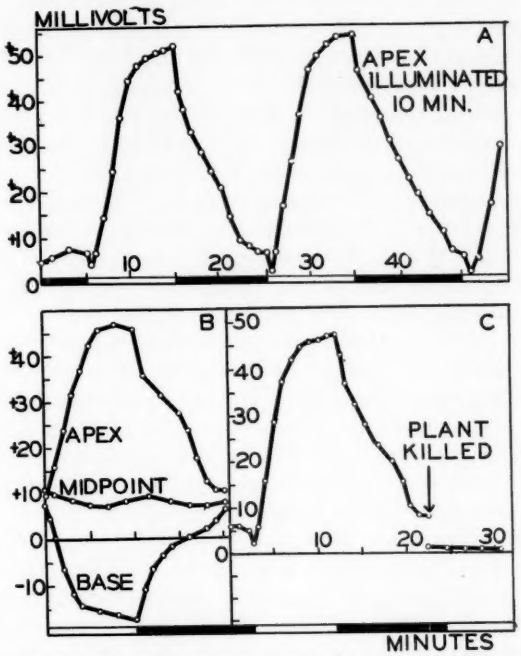

FIG. 3. A. The effect of alternate exposure of the apex to light of 36,500 meter-candles and darkness on the E. M. F. of the first internode. The periods when the light was applied are indicated by the white lines at the base of the drawing.

B. The change in electric polarity of the first internode when different loci along the central axis were illuminated. The curves obtained upon illumination of the different loci are labelled.

C. Absence of the effect of illumination on a dead preparation. The first response was obtained before the plant was killed.

plant's potential during alternate 10-minute exposures of the apex to light and darkness. The illumination for each 10-minutes period was 21.9×10^7 meter-candle-seconds. The periods of darkness are indicated by black bars at the base of the figure, while the illuminated periods are represented by light bars. Illumination produces an immediate decrease, which is followed by an increase of 50 mv. in the potential of the apex with respect to the base. When the light is shut off, there is a drop in potential, and the polarity returns to the original level in darkness. The effect could be repeated a number of times with a duplication in the type of curve, and little variation

in the magnitude of the responses. Within these time limits no evidence of "fatigue" as reported by A. D. WALLER (29) was obtained.

5. *Effect of illumination of different loci along the plant axis.*—Illumination of a given point along the plant axis renders it more positive. When contacts are located at the apex and just below the first node, illumination of the apex causes an increase in the inherent polarity. If the base is illuminated, it becomes more positive. Hence, there follows an inversion of the polarity of the system, which causes the apex to become relatively negative to the base. When the midpoint between apex and base is illuminated there is either no change or a minimum change in the polarity potential. That a change actually occurs in the midregion may be shown by moving the contact to the midpoint and illuminating that area, thereby producing an increase in the local potential at this midpoint. One may further show that a change has occurred by measuring the potential between the apex and midpoint, and returning the contact and measuring the potential. Both methods on the same plant showed an increase of 8 mv. in the illuminated region. This is comparable to the case, discussed earlier in this paper, in which mechanical stimulation produced a decrease of potential at the midpoint but caused no change in the total potential of the internode.

With the claw contact at the midpoint of the first internode, and the water meniscus just below the first node, no change in the potential was obained when the apex was illuminated for 10 minutes. When the claw contact was replaced at the apex of the same plant, and the apex illuminated for 10 minutes, an increase of 42 mv. was obtained. These results indicate that under the conditions of this experiment illumination outside the area of the contacts produces no change in the potential between the contacts.

6. *Effect of killing the preparation on its response to light.*—Death of the plant abolishes all responses to illumination. Figure 3 C shows the normal response of a first internode of a living plant to apical illumination. At the point designated on the graph, the plant was removed from the instrument and immersed for an instant in boiling water. When the plant was replaced in the instrument, with the electrodes at the same position, no change in potential occurred upon apical illumination. Plants killed by chloroform and formaldehyde exhibited no polarity and gave no response to light.

PROCEDURE II. CHANGE IN POLARITY WITH VARIATION OF EXTERNAL FACTORS KNOWN TO AFFECT PHOTOSYNTHESIS

1. *Effect of the intensity of illumination on the electrical polarity of the standard preparation.*—A. Discontinuous illumination of the whole plant with light of varying intensity.—Light from globes of different candle power was reflected through water filters by means of the four symmetrical mirrors

arranged as previously described. The claw contact was at the apex, and the water meniscus contact just below the base of the first node of the standard preparation. Illumination was supplied by different lamps for the same length of time. The periods of illumination are represented by the light bars, and the periods of darkness by the dark bars at the base of figure 4 A.

The plant was illuminated at the points A, B, C, and D in the curve from bulbs of 18-, 90-, 185-, and 240-candle power, respectively, placed one meter above the aquarium. With the lowest intensity of illumination, 18-candle power, at A, little response was obtained. When the lights with increased candle power were used there was an increase in the magnitude of the response. Attention is called to the inversion of polarity which takes place when the light is turned off, which result will be explained later. After each increased response, there is an increase in the magnitude of the inversion. When the plant was allowed to remain in darkness at the end of the experiment, the potential returned to the normal base level.

B. Discontinuous illumination of the apex with light of varying intensity.—Figure 4 B shows the effect of increasing the intensity of apical illumination on the polarity of the standard preparation. The light was applied for 10-minute intervals followed by 10-minute periods in darkness. No change was obtained at A when light of the intensity of 486 meter-candles was used. A change of 20 mv. took place upon illumination with light of 1215 meter-candles. At the points on the graph indicated by D, E, and F, apical illumination of 11,500, 17,100, and 36,500 meter-candles produced responses of 50, 52, and 54 mv. respectively.

Examination of the data shows that there is a lower threshold of light intensity below which no change is produced upon illumination. Above this threshold a relatively small change in luminous intensity produces a large increase in polarity. An increase of the illumination above 17,000 meter-candles produced but little increase in the response of the plant.

C. Continuous illumination of the apex with light of varying intensity. —A potential increase of 5 to 52 mv. produced by illumination with 36,500 meter-candles was obtained between the base and the apex of the first internode of a standard preparation (fig. 4 C). At 10-minute intervals the light intensity was decreased by decreasing the diameter of the effective aperture of the lens. The decrease of illumination at B to 17,100 meter-candles produced no significant decrease in the potential. Reduction of the light-intensity at C to 11,500 meter-candles caused a decrease of 7 millivolts in the polarity. The reduction of the polarity at D, E, and F of 11.5, 15, and 10 mv., respectively, was due to a decrease of the light intensity of 3650, 1215, and 486 meter-candles, respectively. The decrease at G from 486 meter-candles to complete darkness produced no apparent effect. When light was increased, as shown by the ascending part of the graph, there was

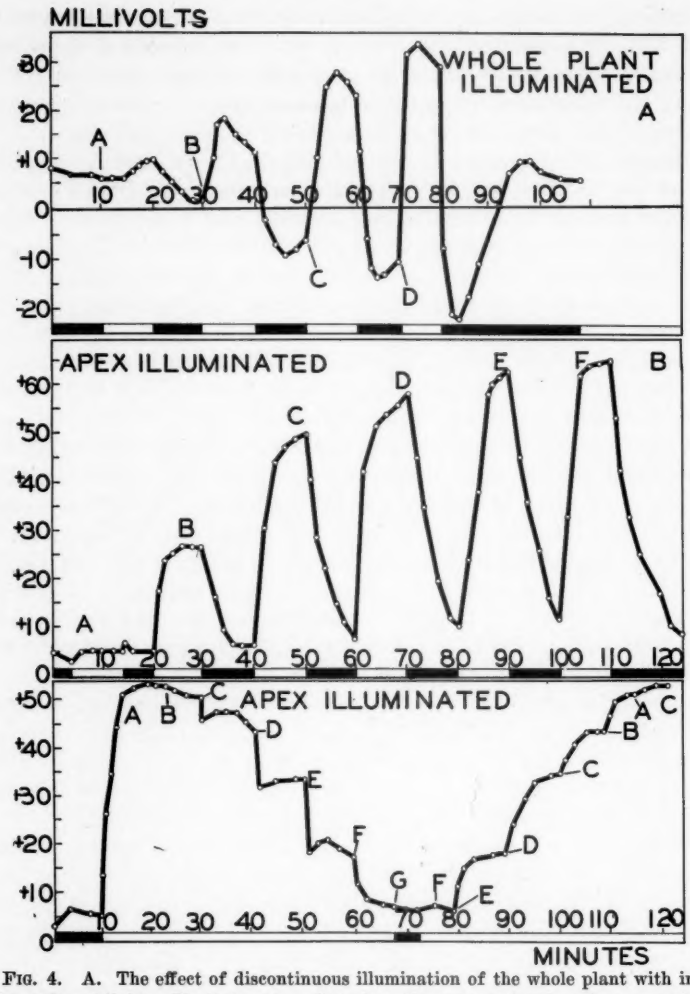

FIG. 4. A. The effect of discontinuous illumination of the whole plant with increasing intensity of light. The light was reflected on the preparation by mirrors on the four sides of the aquarium. Illumination at A was with an 18-c.p. globe at 1 meter, B with 90, C with 185 and D with 240-c.p. globes, respectively.

B. The effect of discontinuous apical illumination of increasing intensity. The apex was illuminated at A with 486 meter-candles, at B with 1215, at C with 3650, at D with 11,500, at E with 17,100, and at F with 36,500 meter-candles, respectively. The periods of illumination are indicated by the unshaded regions of the line at the base of the figure.

C. The control of the magnitude of polarity by decreasing and increasing illumination of the apex. The apex was illuminated with 36,500 meter-candles. At B the intensity of illumination was decreased to 17,100, at C to 11,500, at D to 3650, at E to 1215, at F to 486 meter-candles and at G to complete darkness. In the second half of the curve the illumination was increased by corresponding values.

a corresponding increase in the potential difference of the base to the apex. From these data one may assume that, with other conditions the same, the level of electrical polarity in the plant is largely a function of the intensity of light on the more apical region. By controlling illumination the electric polarity may be decreased to any desired level.

2. *Discontinuous light of different wave lengths.*—Light of different wave lengths was obtained by placing Corning colored glass filters between the plant and the source of illumination, as designated by C in figure 5. The

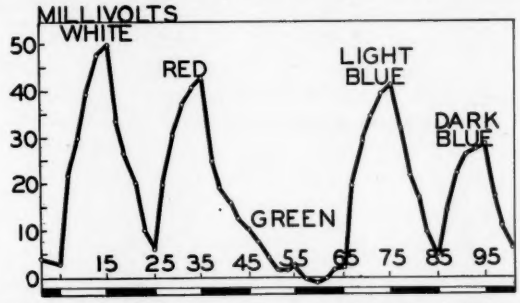

FIG. 5. The effect of stimulation of the apex with light of different wave lengths on the E. M. F. of the first internode. The color of the filter used is stated above each response. The wave length of the light transmitted by the filter marked "red" is 649 to 740 mμ, "green" from 490 to 555 mμ, "light blue" from 445 to 505 mμ, "dark blue" from 415 to 490 mμ. The time of application of light can be ascertained from the unshaded lines at the bottom of the figure.

wave length of the light transmitted by the dark blue filter was from 415 mμ to 490 mμ, by the light blue filter from 445 to 505 mμ, the green filter from 490 mμ to 550 mμ, and the red filter from 649 to 740 mμ.

Light from a tungsten filament lamp filtered through clear glass and falling on the apex of the standard preparation produced a positive increase of 47 mv. labeled "white" in figure 5. Red light of the wave length 649 to 740 mμ produced a change of the same type but only 38 mv. in magnitude. Light passing through the light blue filter (wave length 445 to 505 mμ) produced an increase of 40 mv. and the dark blue (414 to 490 mμ) caused an increase of 22 mv. Green light (490 to 555 mμ) failed to produce any change. Repetition of the above experiment failed in all cases to give an increase in the E. M. F. with green light. These experiments indicate only qualitatively the effect of light of different wave lengths, since neither the intensities nor the light energies present in the light of different wave lengths are comparable.

3. *Effect of CO_2 on the polarity with apical illumination.*—With the light intensity used in these experiments, the concentration of CO_2 was the limiting factor (**29**). An increase in CO_2 in constant illumination should speed

up the rate of photosynthesis, subsequently producing an increase in the potential of the illuminated region, if the rise in intracellular concentration of O_2 is responsible for the increase in magnitude of potential. The effect of CO_2 on the polarity of a standard preparation in darkness in air was first determined. When 1 per cent. CO_2 by volume was introduced into the space above the water level in the aquarium, no effect was produced in the polarity of the plant as shown in figure 6 A. Upon illumination of the apex of a plant in water-vapor saturated air, the potential increased to a maximum;

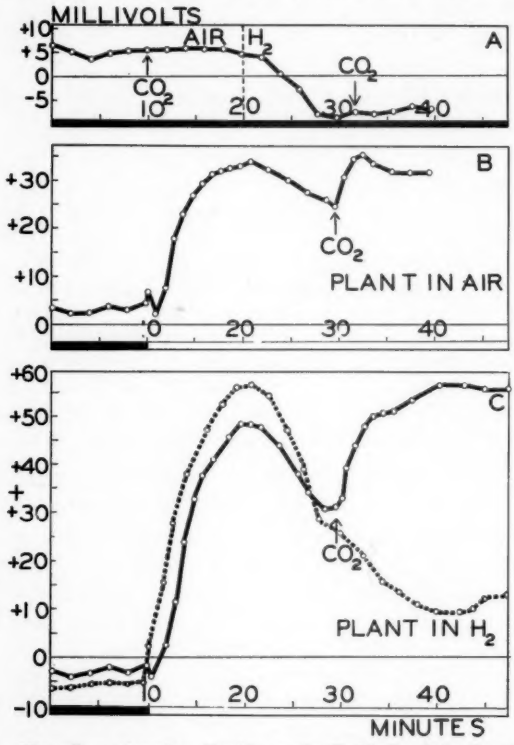

FIG. 6. A. The effect of carbon dioxide on the E. M. F. of the first internode in air and in hydrogen in darkness. The time of application of 1 per cent. carbon dioxide is indicated by the arrows. The dotted line shows the time the gas surrounding the plant was changed from air to hydrogen.

B. The rise of potential on increasing the carbon dioxide concentration in the air surrounding the plant, the apex of which was constantly illuminated. The time of the introduction of 1 per cent. carbon dioxide is indicated by the arrow.

C. The broken curve shows the effect of continuous illumination of the apex in an atmosphere of hydrogen. The solid line represents a comparable preparation under the same condition except that 1 per cent. carbon dioxide is added at the time indicated.

then it fell to a constant level as in the other cases previously discussed. Now, with the introduction of CO_2 gas in light (fig. 6 C), there was in this case an abrupt increase in potential. After the initial overshooting, the potential dropped slightly but was maintained at a new level higher than the original.

The broken curve in figure 6 C shows the electrical behavior of the first internode of a standard preparation in hydrogen, with apical illumination. The plant was allowed to remain 30 minutes in an atmosphere of hydrogen before the first reading was recorded. The initial polarity as shown by the graph was inverted. The initial potential rapidly increased upon illumination to a maximum of 55 mv. This increase was followed by a decrease to a lower level than usually is attained by preparations under the same condition in air. The continuous curve represents a comparable preparation under the same experimental conditions, except that 1 per cent. CO_2 was introduced at the point indicated on the curve. The effect produced was an immediate increase of the potential to a constant high level. Since the rate of photosynthesis depends on the concentration of CO_2, the process is speeded up when the concentration of CO_2 is increased. This increase in photosynthesis evidently raises the concentration of oxygen and other electromotively active substances available for cell oxidation and therefore for the maintenance of electrical polarity.

Procedure III. Effect of oxygen tension on the electrical response to illumination

1. *Effect of oxygen tension on the standard preparation in darkness.*—In this experiment, the claw electrode contact was at the apex and the water meniscus contact at the base of the first internode of the standard preparation. The gases, which were saturated with water vapor by passing them through Bunsen columns filled with distilled water, were passed at a constant rate through the space above the water level in the aquarium. Examination of figure 7 A shows that in hydrogen in darkness a decrease of approximately 20 mv. was obtained with an inversion of polarity. Incidentally, it is impossible to obtain an atmosphere about the plants entirely free of oxygen since dissolved oxygen from the water was continuously passing into the atmosphere above the water. However, in spite of this fact the results are perfectly clear. When oxygen was introduced, there was a subsequent increase of 30 mv. in the polarity potential, followed by a decrease and a subsequent rise to a constantly maintained level of potential. Repetition on several different plants gave essentially the same results. These curves are strikingly similar to those obtained on the effects of presence and absence of oxygen on the potential in the onion root and may be interpreted in the same manner (Rosene and Lund, **25**).

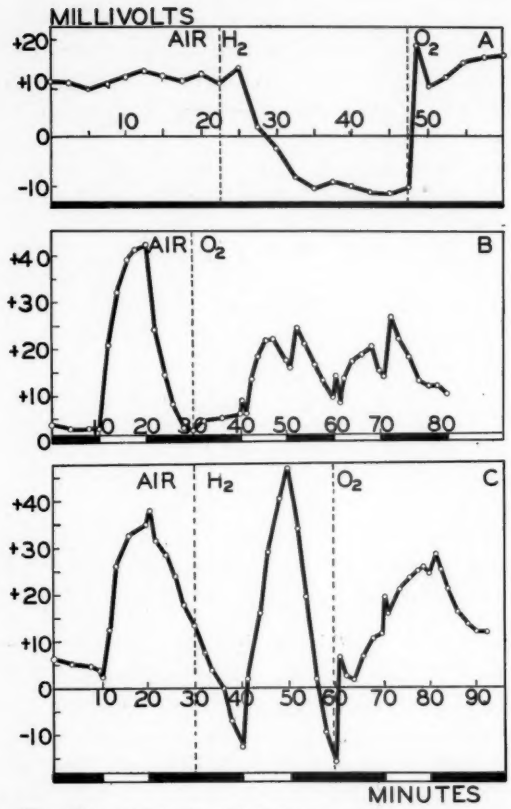

FIG. 7. A. The effect of (a) air, (b) hydrogen, and (c) oxygen on the polarity of the standard preparation in darkness. The times of change of the gases are shown by the dotted lines.

B. The response produced by illuminating the apex of the standard preparation in atmospheres of air and oxygen. The time of application of the light may be determined from the unshaded portions of the line at the base of the plate. Oxygen was introduced at the time indicated by the dotted line.

C. The change in E. M. F. of the standard preparation produced by apical illumination of the standard preparation in atmospheres of air, hydrogen, and oxygen. The period of illumination is indicated by the unshaded portions of the line at the base of the drawing. The broken line shows the time of change of gases.

2. *Comparison of the modifications of the response due to illumination in an atmosphere of oxygen, hydrogen, and air.*—Figure 7 B shows the effect of illumination on the standard preparation, first in air and then in oxygen, the intensity and time of the illumination being the same in both cases. A

typical response of 42 mv. was obtained with the plant in air. When the atmosphere about the plant, in darkness, is changed from air to oxygen, there is a small increase in potential. Illumination in oxygen causes an initial rise and fall of only 2 mv., followed by a potential increase. The potential reaches a maximum and then decreases. Upon discontinuing the illumination there is an immediate rise in the potential followed by a decrease to a lower level than in light. In air, there is an increase in potential of 41 mv. compared to 15 and to 9 in oxygen.

Figure 7 C shows the effect of light on the apex of the standard preparation in an atmosphere of air, pure hydrogen, and pure oxygen. In air, the illumination produced an increase of 35 mv. in the polarity of the first internode. In hydrogen the same illumination produced an increase of 59 mv., while in oxygen only a 15 mv. response was obtained. *Attention is called to the decrease in potential when hydrogen replaced the air in darkness, and subsequent increase in potential when oxygen replaces the hydrogen in darkness.*

Discussion

The purpose of the first part of this investigation was to establish a relation between light and the maintained polarity in *Chara*. The change upon illumination cannot be attributed to an increase in temperature, as the heat rays are filtered out by the water. The ultra violet rays are removed by the glass. Therefore any change must be due to the effect of light of the visible spectrum. Visible light could affect either the photosynthetic process or some unknown photoreceptive mechanism in the plant.

An increase in the intensity of illumination increases the electric polarity.[1] An increase in CO_2 concentration in light increases the electric potential. Green light produces no change in the E. M. F. of the system, while red and blue light both caused an increase in the potential. Likewise, in green plants (29) the increase in intensity of illumination and the concentration of CO_2 increases the rate of photosynthesis; also, in green light photosynthesis is at a minimum. Since all these facts are consistent with one another, one may logically conclude that photosynthesis is closely linked with the increase in the electrical potential due to illumination. Furthermore, from the following facts, it becomes practically certain that the effects of light on the E.

[1] The electrical polarity of a single cell is

$$E_p = -RT/2F \ln Pc_1/Pc = -RT/2F \ln r',$$

where R is the gas content, T, the absolute temperature, ln, the natural logarithm, F, the faraday equivalent, and Pc the oxygen pressure at a given locus inside the cell, and r′ is the ratio of Pc_1 to Pc. The electrical polarity of a polar tissue may be expressed as:

$$E = \Sigma E = -RT/2F \ln r' \text{ apical}/r' \text{ basal}.$$

Pc has been shown to be quantitatively related to P, the pressure of oxygen surrounding the tissue.

M. F.'s are primarily caused by changes produced in the cell by photosynthesis.

1. Oxygen is released by photosynthesis.
2. A quantitative relation exists between the output of electric energy and cell oxidation in all polar tissues which have been investigated up to the present.
3. The quantitative relations between oxygen tension, velocity of O_2 consumption, and electric potential in the onion root have been formulated by LUND (**17**) on the basis of flux equilibrium in cell oxidation.

Using LUND'S fundamental assumptions (**12**) as a basis for his work MARSH has recently extended this formulation, along with direct experimental evidence, to show how oxygen concentration is related to the inherent E. M. F. In such a system the loci may be considered to act essentially as modified oxygen electrodes. The above conclusions of course do not exclude the possibility that other products of photosynthesis, besides free oxygen, also affect the potential.

The rate of production of oxidizable substance, which is equivalent to the oxygen consumption at flux equilibrium, has been shown to be greater in the apical than in the basal regions of various tissues (**12, 17, 21**). In view of this fact, we should expect the increase in potential at the locus of the apex, upon illumination of the apex, to be greater than that in the base. This is precisely what was observed in the experiments on the illumination of the whole plant (fig. 2 B), and with illumination of the apex and base separately (fig. 3 B).

The inversion of polarity (fig. 4 A) after illumination of the whole preparation can be explained by the difference in the rate of oxygen consumption of the apex and the base. If O_2 were produced by photosynthesis at approximately equal rates at both apex and base, an unequal change in the potential at the two loci would result, since it has been found that the same change in concentration of oxygen at apex and base causes a greater change in the apical than in the basal tissues (**26**). The increased consumption of oxygen, continuing at the apex after the light is removed, would cause a relative decrease in the electrical potential. Since the oxygen is consumed at a lesser rate in the base, it would be available for a longer period of time with the consequent maintenance of a relatively higher potential. The higher positive potential at the base would result in the observed inversion of the polarity of the internode (fig. 4 B). As the oxygen is consumed in the cell oxidations, the polarity gradually reverts, reaching the constant potential level in darkness.

This conception adequately explains the increase in the electrical response in the absence of oxygen. A decrease in oxygen tension around the polar

tissue leads to an inversion of polarity which is in accord with the present observation (fig. 13 A) and with the results of ROSENE and LUND (25) and with MARSH's mathematical formulation (19) of the relation between the flux equilibrium oxygen pressure, Pe, and the oxygen pressure, P, of the atmosphere surrounding the plant. In low concentrations of oxygen as all the available oxygen is used up by respiration of the plant, the potential is decreased at both the apical and basal loci, the potential of the apex being decreased relatively more than at the base. Upon apical illumination the potential of the apex is again increased relatively more than that of the base, thus accounting for the increase in the apical-basal potential. The potential of the apex apparently decreases (as illustrated in the dotted line of figure 6 C) because of the decrease in the photosynthetic rate due to the removal of CO_2.

Even with the plant in oxygen, Pe does not necessarily equal P. Moreover, upon illumination Pe might be further increased. With the plant in oxygen, the absolute value of the apical Pe and the basal Pe are greater; hence, an increase in the apical Pe up to its maximum does not change the ratio of the apical Pe/basal Pe as much as when the basal flux equilibrium oxygen pressure has a lower value. This is demonstrated in figure 7 B. The method used here may serve to distinguish Pe from P.

Even though the general results obtained on *Chara* may be explained as the effect of an increased production of oxygen in the polar tissues, it is probable that the observed changes in the electrical potential may also be partly determined by other concomitant electrochemical processes in the cell. CLARK (5) and WILKS (*loc. cit.*) report definite electrical changes due to illumination in the etiolated oat coleoptile which, in the absence of chlorophyll, are not due to photosynthesis. WALLER reports "photoelectric" changes in the etiolated petals of some plants. It is also highly probable that electromotively active substances other than oxygen are produced in the photosynthetic process.

The experimental results have shown that light when appropriately applied may act differentially and quantitatively to increase and decrease reversibly the electric polarity and the potential at any locus on the main axis in *Chara*. In this respect the effect of light is similar to the effect of temperature, and of the concentration of oxygen around the tissue, both of which are factors influencing cell oxidation. The output of electrical energy appears to depend on the flux equilibria between the substances necessary for cell oxidation and the concentration of oxygen. Both of these may act as limiting factors. By photosynthesis under certain conditions the chloroplasts supply the oxygen necessary for the intracellular oxidation and the maintenance of the electrical polarity of the cell.

Summary

1. An apparatus is described for measuring the electrical polarity of *Chara vulgaris* under a minimum of mechanical disturbance.

2. The apex of *Chara vulgaris* is electropositive in the external circuit to the more basal regions. In the unstimulated condition the apex remains positive over a period as long as 48 hours.

3. The slight pressure exerted by a glass contact supporting a plant by the apex under water causes an inversion of the electric polarity. The apex returns slowly to the electropositive condition when the contact is removed.

4. Mechanical stimulation causes the E. M. F. of the stimulated region to become more negative. The change in E. M. F. produced is reversible, returning to the unstimulated condition in about two minutes. Stimuli applied outside the area between the electrode contacts may produce a change in the potential in the region of the plant included between the contacts. No evidence of transmission of the electrical effect of stimulation across the node was obtained.

5. Illumination of the whole plant increases the polarity of the apical region relatively more than the basal regions. The electropositivity of all illuminated regions is increased. The electric response to illumination does not occur in a dead plant.

6. The following facts relate the change of potential upon illumination to photosynthesis: (a) increase of the response with increased intensity of illumination; (b) failure to obtain an increase in potential on illumination with green light, while an increase was obtained in red and blue light; (c) an increase in polarity in light and absence of O_2 with an increase in the carbon dioxide concentration.

7. In low oxygen tension, the response to light is increased; in high oxygen tension it is decreased.

8. The results can be explained on the basis of the equations formulated by MARSH (**22**) for the relation of the flux equilibrium oxygen tension and electric polarity.

The author is grateful to Dr. E. J. LUND, under whose direction this work was carried out, for many helpful suggestions made by him during the course of this investigation.

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A STUDY OF MINERAL NUTRITION OF WHEAT AS INFLUENCED BY FERTILIZER COMBINATIONS[1]

FRANK T. DONALDSON

(WITH TEN FIGURES)

Introduction

Practically the only method of following the movement of mineral nutrients into field crops consists of measuring the actual weights of the various elements present at progressive stages of growth. To do this it becomes necessary to determine not only the percentage composition of the plant, but also the average weight per plant or per tiller. This type of procedure was followed in the investigation described in this paper in an effort to determine the effect of various fertilizer combinations upon the rates of assimilation and the amounts of mineral nutrients entering the wheat plant.

There are comparatively few instances of the application of progressive development studies to wheat in which the whole plant has been studied. One of the earliest investigations of this type was published by WILFARTH, RÖMER, and WIMMER (15) in 1903. In this work samples were gathered from a field of spring wheat on four successive dates and analyses made for starch, potash, sodium, nitrogen, and phosphorus. The data were expressed as percentage of dry matter and pounds per acre.

In 1931 KNOWLES and WATKIN (9) published a thorough and comprehensive investigation of the nutrition of winter wheat. Nine successive samples were gathered over a period extending from seven weeks before ear emergence until harvest. The value of their data is enhanced by the large sample of 3200 tillers that was gathered at each sampling date. From the fourth to the ninth samplings the heads were separated from the leaves and stems and a further division was made of the eighth and ninth samplings into grain and chaff. Their results are expressed as the weights of substances present in 3200 tillers.

While the present investigation was in progress WOODFORD and MCCALLA (16) published the results of their investigation of the nutrition of spring wheat grown on the gray and black soils of Alberta. Their method of procedure was essentially the same as that of the English workers, although their results are expressed as the weights of nutrients present in plants taken from a unit length of row. Since the black soil contained four times the nitrogen and twice the phosphorus of the gray soil, their study, in part,

[1] Contribution from Montana State College, Agricultural Experiment Station. Paper no. 98, Journal Series.

demonstrates the effect of varying amounts of mineral nutrients upon the nutrition of the wheat plant and parallels more closely the work described in the present investigation.

Materials and methods

Conditions under which crop was grown

A plot of ground was selected for this investigation in a locality known to possess rather infertile soil, in order that the response to the added fertilizers might be accentuated. This soil was a light, sandy loam, low in organic matter and total nitrogen and deficient in phosphorus. An analysis of the soil is given in table I.

TABLE I

Chemical analyses of soil upon which wheat was grown

Substance in soil	First foot	Second foot
Phosphorus (percentage)	0.061	0.063
Calcium oxide (percentage)	1.32	5.40
Total nitrogen (percentage)	0.059	0.042
Available phosphorus (Winogradsky test)	deficient	.....
Nitrate nitrogen (p.p.m.)	3.00	6.00

The ground chosen for the plots was planted to potatoes the previous year. The potatoes were never irrigated and, since they received only one cultivation late in the summer, the weeds made a dense growth. While this condition made necessary considerable weed pulling the following year, it no doubt insured the removal of most of the nitrates from the soil, thus providing an opportunity to study the effect of adding nitrates.

A detailed account was kept of weather conditions at the site of the plots and these observations are augmented by the data from the cooperative weather observation station at Bozeman, 22 miles distant. Table II lists the pertinent weather and crop data through the growing season and the dates upon which sample collections were made.

Six one-twentieth-acre plots were seeded. Five of these were fertilized with the various fertilizer combinations shown in table III and one was left unfertilized for a check.

The potassium chloride was of the strength known as 50 per cent. K_2O; the sodium nitrate contained 15 per cent. nitrogen; and the treble superphosphate 43 per cent. available P_2O_5.

Sampling

In all cases samples were collected (table II) before ten o'clock in the morning to avoid the effects of any rhythmic variation in nutrient assimilation which might occur during the day. Plants were pulled from the rows in the

TABLE II

WEATHER AND CROP DATA

DATE OF COLLECTION	WEATHER AND CROP DATA THROUGHOUT GROWING SEASON
1936	
March 1–May 1	The precipitation record at Bozeman shows 1.6 inches for this period compared with the 36-year average of 2.9 inches
May 2–May 13	A good rain fell in the vicinity of the plots on May 7. Bozeman Station recorded 0.85 inch
	On May 13 mineral fertilizers were applied to the plots and Marquis wheat seeded
May 14–June 25	Between May 15 and May 19 there were general rains which insured good germination of the seed
	On May 22 the wheat was just up and appeared in excellent condition
	Rain on June 1, 2, 3, 5, 6, and 7. Rains on June 5, 6, and 7 represented the last precipitation of any benefit during remainder of growing season
	On June 16 wheat in all plots stood 3 to 4 in. high and soil was moist to a depth of 18 in.
	On June 25 plots were sampled for first time. Wheat stood 6 to 10 in. high
June 26–July 6	Hot, dry weather during this period evaporated soil moisture at a rapid rate
	Plots were sampled for second time on July 6. Although the ground was quite dry the plants appeared to be in excellent condition. None of wheat had started to head
July 7–July 16	An attempt was made to irrigate the plots but the demand for water on the farms with prior water rights was so great that there was not sufficient head to raise the water into the field ditch
	On July 16 wheat was sampled for the third time. Wheat on all plots had headed out and showed some damage from the dry weather
July 17–July 24	On July 21, when the demand for water had lessened somewhat, plots were irrigated. Three days later, July 24, the fourth set of samples was collected
July 25–July 31	Fifth set of samples was collected on July 31. The kernel was in the firm dough stage at this time
Aug. 1–Aug. 7	On August 7 the sixth and last set of samples was gathered. Wheat was ready to harvest and was cut the following day

plots in one-foot sections in such a manner that the evacuated spaces formed a series of steplike patterns in the plot. No plants were used from the two outside rows nor from three-foot sections at the ends of the plots.

TABLE III

FERTILIZERS APPLIED TO THE ONE-TWENTIETH-ACRE PLOTS

PLOT NO.	KINDS AND AMOUNTS OF FERTILIZERS
1	None
2	10 pounds KCl, 12 pounds $NaNO_3$, 20 pounds treble superphosphate
3	10 pounds KCl, 20 pounds treble superphosphate
4	20 pounds treble superphosphate
5	12 pounds $NaNO_3$
6	10 pounds KCl, 12 pounds $NaNO_3$

Only an approximate count was made of the plants at the time of sampling, but an effort was made to have the sample contain from 200 to 300 plants. As soon as each collection was completed, the roots were wrapped in moist cheese cloth and the samples transported to the laboratory at Bozeman. Upon arrival at the laboratory the plants were counted and the roots cut from the plant one inch above the ground level and discarded. In the last three samples the heads were separated from the leaves and stems for separate analysis. The samples were then placed in a large oven held at 65° C. and equipped with a forced air draft. After drying for 24 hours the samples were removed to the open room and allowed to come to equilibrium with the moisture of the atmosphere. They were then weighed and ground in a Wiley mill to pass a 1-mm. screen.

Analyses

Dry matter.—Moisture determinations were made on the air-dried material. The samples were held 5 hours in a vacuum oven at 98° C. The percentages of all the nutrients determined are based on the dry matter as determined by this procedure.

Nitrogen.—Total nitrogen was determined by the Kjeldahl method with metallic mercury as a catalyst. A modification of the reduced iron method of Pucher, Leavenworth, and Vickery (**12**) was used on the samples collected at the first two sampling dates to include nitrate nitrogen.

Phosphorus.—Phosphorus was determined gravimetrically by precipitation as magnesium ammonium phosphate after first digesting the sample in a mixture of perchloric and nitric acids (**5, 6**).

Sulphur.—An aliquot of the perchloric-nitric acid digest was neutralized with ammonia, made slightly acid with hydrochloric acid, and the sulphates precipitated with barium chloride.

Potassium.—Potassium from a third aliquot of the acid digest was determined by the perchloric acid method (**7, 8**).

Ash.—The sample was burned over night in a platinum dish at a temperature of approximately 560° C.

Soluble ash.—Soluble ash was determined as that part of the ash soluble in N/2 hydrochloric acid.

Presentation of results

A unit of one plant was chosen as the basis for calculating the results of this study. The total dry weight of each sample was divided by the number of plants in the sample to obtain the average weight per plant. The product of this value and the percentage composition is expressed in milligrams per plant for all data. The term "whole plant" is used to designate the entire aerial portion of the plant. Since the heads were not counted, the data given for heads are based on the average weight of heads per plant.

TABLE IV

AVERAGE AMOUNTS OF SUBSTANCES IN ENTIRE AERIAL PORTION OF PLANT EXPRESSED AS MILLIGRAMS PER PLANT

PLOT	FERTILIZER TREATMENT	SUBSTANCES IN PLANT ON DATE OF SAMPLING					
		JUNE 25	JULY 6	JULY 16	JULY 24	JULY 31	AUG. 7
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
		Dry matter					
1	Check	433	1080	1440	1620	1790	1910
2	NPK	584	1240	1730	1970	2110	2250
3	PK	521	1080	1510	1820	2030	2060
4	P	562	1270	1630	1920	2150	2280
5	N	507	1070	1480	1790	1970	2110
6	NK	431	1000	1520	1720	1990	1970
		Phosphorus					
1	Check	1.18	2.54	2.64	2.82	3.14	3.69
2	NPK	1.65	2.86	3.06	3.50	3.50	3.91
3	PK	1.42	2.59	2.93	3.10	3.57	3.84
4	P	1.53	3.05	3.18	3.12	3.76	4.37
5	N	1.27	2.21	2.50	2.84	3.10	3.57
6	NK	1.12	2.11	2.40	2.91	3.22	3.22
		Sulphur					
1	Check	1.25	2.93	3.11	3.35	3.42	3.70
2	NPK	1.52	2.96	3.18	3.53	3.57	3.78
3	PK	1.30	2.65	2.88	3.41	3.47	3.65
4	P	1.55	3.35	3.31	3.64	3.90	4.10
5	N	1.43	2.70	2.84	3.46	3.47	3.95
6	NK	1.15	2.42	2.78	3.28	3.65	3.85
		Nitrogen					
1	Check	15.0	25.7	25.8	25.0	26.5	27.1
2	NPK	20.8	28.9	30.6	31.6	31.0	32.9
3	PK	17.5	24.9	26.6	28.2	30.1	29.3
4	P	19.1	29.5	28.9	30.6	31.6	32.6
5	N	18.9	27.3	27.0	28.8	29.4	30.9
6	NK	16.1	25.0	27.2	27.4	29.7	28.2
		Calcium					
1	Check	1.99	3.63	3.50	3.58	3.87	3.86
2	NPK	2.59	4.10	3.93	4.63	4.43	4.62
3	PK	2.28	3.56	3.37	3.99	4.05	4.14
4	P	2.59	4.62	4.08	4.56	4.49	4.45
5	N	2.32	3.43	3.64	4.16	4.33	4.21
6	NK	2.02	3.03	3.63	3.73	3.92	3.79
		Potassium					
1	Check	19.0	37.0	30.2	26.6	22.3	21.4
2	NPK	28.1	44.4	36.2	34.2	26.6	27.3
3	PK	22.1	35.2	29.6	29.4	24.2	23.8
4	P	23.9	41.8	31.0	30.2	25.5	24.5
5	N	22.6	38.7	31.4	29.1	25.8	25.1
6	NK	19.6	36.3	32.1	28.8	28.8	25.7

TABLE IV—(*Continued*)

Plot	Fertilizer treatment	Substances in plant on date of sampling					
		June 25	July 6	July 16	July 24	July 31	Aug. 7
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
		Soluble ash					
1	Check	38.4	74.2	61.8	58.9	53.1	54.7
2	NPK	55.5	89.7	74.0	76.8	63.9	69.2
3	PK	47.6	86.0	58.9	64.6	60.7	62.2
4	P	52.0	88.1	67.2	68.3	62.5	64.5
5	N	51.5	89.0	66.0	70.3	62.8	63.4
6	NK	42.9	73.1	66.7	65.2	66.7	62.6
		Ash					
1	Check	62.7	129	137	162	201	190
2	NPK	86.5	149	161	198	214	223
3	PK	79.5	141	148	187	212	213
4	P	76.3	148	156	191	206	228
5	N	78.7	132	141	173	218	216
6	NK	72.5	128	161	167	196	191

The data for the development of the whole plant are presented first. The results for all the plots are discussed collectively under a separate heading for each substance determined. After the data are presented from the standpoint of the plant as a whole, the distribution of the nutrients between heads and straw is discussed. The term straw is used to include all that portion of the plant, with the exception of the heads, lying above the ground level.

Experimentation

Progressive development of the whole plant upon application of various nutrients

All the data for the average weights of substances per plant at the successive stages of growth (table IV), the corresponding percentage of these substances in dry matter (table V), and the amounts of substance in the whole plant at the various stages of growth as percentages of the maxima (table VI) are given in the following discussion.

Dry Matter

The data for dry matter are presented graphically in figure 1. The average weight per plant on all plots except no. 6 increased from the first sampling to the last. The weights of plants on plot 6 reached a maximum the week before harvest and remained constant during the last week. Plots 2 and 4, whose fertilizer treatments were complete and phosphorus alone, respectively, yielded plants of greater weight through the entire growing

season. The plants on the check plot had approximately the same weight as those on plots 3, 5, and 6 for the first three samplings. Subsequently, the plants from the three fertilized plots gradually showed a slight increase over those from plot 1.

It is apparent that phosphorus had the most beneficial effect on growth of any of the three nutrients added. When potassium was added with

TABLE V

AVERAGE PERCENTAGE OF SUBSTANCES IN DRY MATTER OF ENTIRE AERIAL PORTION OF PLANT

PLOT	FERTILIZER TREATMENT	SUBSTANCES IN DRY MATTER ON DATE OF SAMPLING					
		JUNE 25	JULY 6	JULY 16	JULY 24	JULY 31	AUG. 7
		%	%	%	%	%	%
		Phosphorus					
1	Check	0.273	0.235	0.183	0.174	0.175	0.190
2	NPK	0.282	0.231	0.177	0.178	0.166	0.174
3	PK	0.272	0.240	0.194	0.170	0.176	0.186
4	P	0.272	0.240	0.195	0.163	0.175	0.192
5	N	0.250	0.207	0.169	0.159	0.157	0.169
6	NK	0.259	0.211	0.158	0.169	0.162	0.163
		Sulphur					
1	Check	0.289	0.271	0.216	0.207	0.191	0.194
2	NPK	0.261	0.239	0.184	0.179	0.169	0.168
3	PK	0.249	0.245	0.191	0.187	0.171	0.177
4	P	0.275	0.264	0.203	0.190	0.181	0.180
5	N	0.282	0.252	0.192	0.193	0.176	0.187
6	NK	0.266	0.242	0.183	0.191	0.183	0.195
		Nitrogen					
1	Check	3.47	2.38	1.79	1.54	1.48	1.42
2	NPK	3.57	2.33	1.77	1.60	1.47	1.46
3	PK	3.36	2.31	1.76	1.55	1.48	1.42
4	P	3.40	2.32	1.77	1.59	1.47	1.43
5	N	3.73	2.55	1.82	1.61	1.49	1.46
6	NK	3.74	2.50	1.79	1.59	1.49	1.43
		Calcium					
1	Check	0.460	0.336	0.243	0.221	0.216	0.202
2	NPK	0.443	0.331	0.227	0.235	0.210	0.206
3	PK	0.438	0.330	0.223	0.219	0.200	0.201
4	P	0.461	0.364	0.250	0.238	0.209	0.195
5	N	0.458	0.321	0.246	0.232	0.220	0.200
6	NK	0.468	0.303	0.239	0.217	0.197	0.192
		Potassium					
1	Check	4.38	3.43	2.10	1.64	1.25	1.11
2	NPK	4.82	3.58	2.09	1.74	1.26	1.21
3	PK	4.24	3.26	1.96	1.62	1.19	1.16
4	P	4.25	3.29	1.90	1.57	1.19	1.07
5	N	4.46	3.62	2.12	1.63	1.31	1.19
6	NK	4.55	3.63	2.11	1.67	1.45	1.30

TABLE V—(*Continued*)

Plot	Fertilizer treatment	Substances in dry matter on date of sampling					
		June 25	July 6	July 16	July 24	July 31	Aug. 7
		%	%	%	%	%	%
		Soluble ash					
1	Check	8.86	6.87	4.29	3.64	2.97	2.86
2	NPK	9.50	7.23	4.28	3.90	3.03	3.08
3	PK	9.14	7.96	3.90	3.55	2.99	3.02
4	P	9.25	6.94	4.12	3.56	2.91	2.83
5	N	10.2	8.24	4.46	3.93	3.19	3.00
6	NK	9.95	7.31	4.39	3.79	3.35	3.18
		Ash					
1	Check	14.5	11.9	9.54	10.0	11.2	9.95
2	NPK	14.8	12.0	9.30	10.1	10.1	9.91
3	PK	15.3	14.0	9.82	10.3	10.4	10.3
4	P	13.6	11.7	9.54	9.95	9.58	10.0
5	N	15.5	12.3	9.56	9.66	11.1	10.2
6	NK	16.8	12.8	10.6	9.71	9.85	9.70

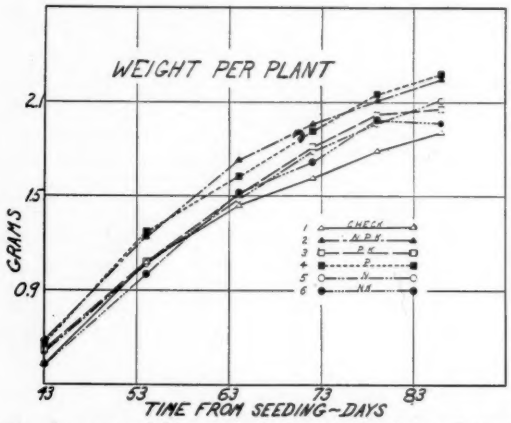

Fig. 1. Average weight per plant at successive dates of sampling.

phosphorus, the plants made a poorer growth than with phosphorus alone, but when a nitrate fertilizer was added in addition, as on plot 2, the deleterious effect of the potassium fertilizer was offset. It will be noted, however, that plot 4, treated only with treble superphosphate, supported just as heavy growth as plot 2, fertilized with all three nutrients.

Since the uptake of mineral nutrients takes place at a rapid pace early in the growth of the wheat plant, it might be expected that a readily available supply of all nutrients would favor a more rapid development of the

TABLE VI

AVERAGE AMOUNTS OF SUBSTANCES PRESENT IN AERIAL PORTION OF PLANT AT VARIOUS DATES OF SAMPLING EXPRESSED AS PERCENTAGES OF THE MAXIMA

PLOT	FERTILIZER TREATMENT	AV. AMOUNT OF SUBSTANCES ON DATES OF SAMPLING					
		JUNE 25	JULY 6	JULY 16	JULY 24	JULY 31	AUG. 7
		%	%	%	%	%	%
		Dry matter					
1	Check	22.7	56.5	75.4	84.8	93.7	100.0
2	NPK	26.0	55.1	76.9	87.6	93.8	100.0
3	PK	25.3	52.4	73.3	88.3	98.5	100.0
4	P	24.6	55.7	71.5	84.2	94.3	100.0
5	N	24.0	50.7	70.1	84.8	93.4	100.0
6	NK	21.7	50.3	76.4	86.4	100.0	99.0
		Phosphorus					
1	Check	32.0	68.8	71.5	76.4	85.1	100.0
2	NPK	42.2	73.1	78.3	89.5	89.5	100.0
3	PK	37.0	67.4	76.3	80.7	93.0	100.0
4	P	35.0	69.8	72.8	71.4	86.0	100.0
5	N	35.6	61.9	70.0	79.6	86.8	100.0
6	NK	34.8	65.5	74.5	90.4	100.0	100.0
		Sulphur					
1	Check	33.8	79.2	84.1	90.5	92.4	100.0
2	NPK	40.2	78.3	84.1	93.4	94.4	100.0
3	PK	35.6	72.6	78.9	93.4	95.1	100.0
4	P	37.8	81.7	80.7	88.8	95.1	100.0
5	N	36.2	68.3	71.9	87.6	87.8	100.0
6	NK	29.9	62.9	72.2	85.2	94.8	100.0
		Nitrogen					
1	Check	55.3	94.8	95.2	92.3	97.8	100.0
2	NPK	63.2	87.8	93.0	96.0	94.2	100.0
3	PK	58.1	82.7	88.4	93.7	100.0	97.3
4	P	58.6	90.5	88.7	93.9	96.9	100.0
5	N	61.2	88.3	87.4	93.2	95.1	100.0
6	NK	54.2	84.2	91.6	92.3	100.0	94.9
		Calcium					
1	Check	51.4	93.8	90.4	92.5	100.0	99.7
2	NPK	55.9	88.6	84.9	100.0	95.7	99.8
3	PK	55.1	86.0	81.4	96.4	97.8	100.0
4	P	56.1	100.0	88.3	98.7	97.2	96.3
5	N	53.6	79.2	84.1	96.1	100.0	97.2
6	NK	51.5	77.3	92.6	95.2	100.0	96.7
		Potassium					
1	Check	51.4	100.0	81.6	71.9	60.3	57.8
2	NPK	63.3	100.0	81.5	77.0	59.9	61.5
3	PK	62.8	100.0	84.1	83.5	68.8	67.6
4	P	57.2	100.0	74.2	72.2	61.0	58.6
5	N	58.4	100.0	81.1	75.2	66.7	64.9
6	NK	54.0	100.0	88.4	79.3	79.3	70.8

TABLE VI—(*Continued*)

Plot	Fertilizer treatment	Av. amount of substances on dates of sampling					
		June 25	July 6	July 16	July 24	July 31	Aug. 7
		%	%	%	%	%	%
		Soluble ash					
1	Check	51.8	100.0	83.3	79.4	71.6	73.7
2	NPK	61.9	100.0	82.5	85.6	71.2	77.1
3	PK	55.3	100.0	68.5	75.1	70.6	72.3
4	P	59.0	100.0	76.3	77.5	70.9	73.2
5	N	57.9	100.0	74.3	79.0	70.6	71.2
6	NK	58.7	100.0	91.2	89.2	91.2	85.6
		Ash					
1	Check	31.2	64.2	68.2	80.6	100.0	94.5
2	NPK	38.8	66.8	72.2	88.8	96.0	100.0
3	PK	37.3	66.2	69.5	87.8	99.5	100.0
4	P	33.5	64.9	68.4	83.8	90.4	100.0
5	N	36.1	60.6	64.7	79.4	100.0	99.1
6	NK	37.0	65.3	82.1	85.2	100.0	97.4

plant during the early stages of growth. This is demonstrated in the case of plot 2 where the plants had a higher percentage of their maximum weight present at the first sampling than the plants of any of the other plots. The plants from plots 1 and 6 had a lower percentage of their maximum development present at the first sampling than the plants of the other plots.

Phosphorus

The data for phosphorus are presented graphically in figure 2. The percentage of phosphorus of the plants of all plots showed a sharp decline to the third and fourth samplings when a slight increase took place. The plants from the three plots to which nitrate was added had the lowest percentages on the final sampling dates. The effect of phosphorus upon the percentage nitrogen is a well established fact and has been noted by many workers (**1, 11**), but the reciprocal relationship is not so well established. It must be observed, however, that at the first sampling the plants from plot 2 had the highest percentage phosphorus of the six, indicating that when ample phosphorus is available, nitrates do not exert this depressing effect during the early periods of rapid assimilation.

It was pointed out in the previous section that plot 3, fertilized with phosphorus and potassium, produced plants of lower weight than did plot 4, fertilized with phosphate alone. That this deleterious effect was not due to a depressing effect of potassium upon phosphorus assimilation is shown by the percentage data in figure 2. The percentages of phosphorus in the plants from plots 3 and 4 were nearly identical from the first sampling to the last, and both were comparatively high.

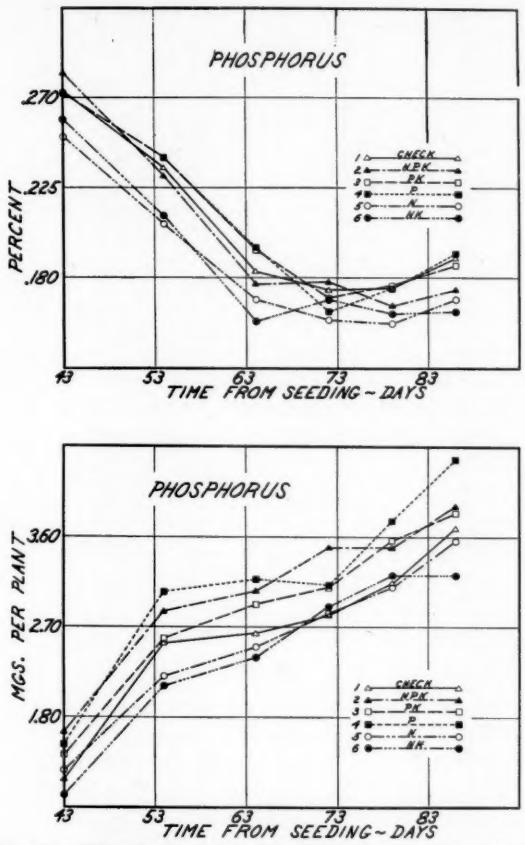

FIG. 2. Upper graph, phosphorus as percentage of dry matter; lower graph, phosphorus as milligrams per plant.

In all cases the maximum amount of phosphorus was present in the plants at the last sampling and the plants from all plots, except plot 6, showed a considerable increase between the last two samplings. This is at variance with the findings of both the German and the English workers.

SULPHUR

The data for sulphur are presented graphically in figure 3. Between the first and second samplings the percentage of sulphur of the plants from all the plots showed a slight decrease. Between the second and third samplings the plants from all the plots again followed similar trends and

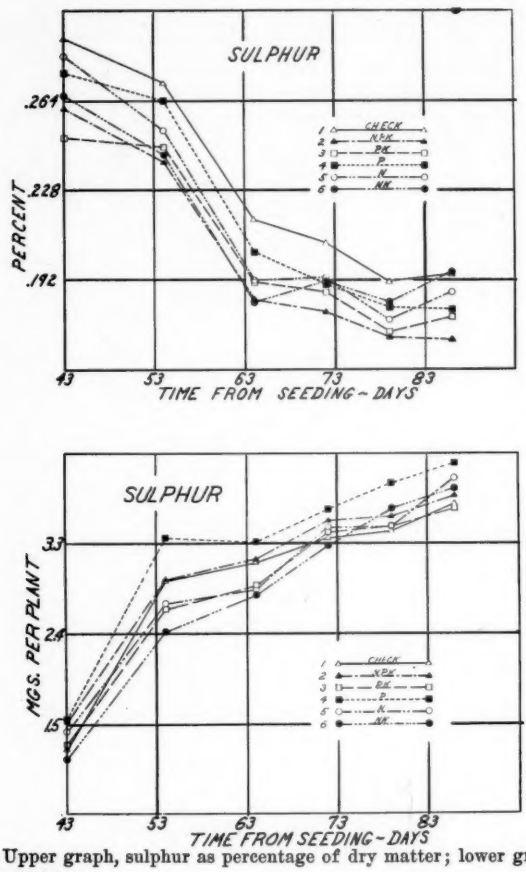

FIG. 3. Upper graph, sulphur as percentage of dry matter; lower graph, sulphur as milligrams per plant.

decreased sharply. With one or two exceptions there was once more a slow decrease in the percentage of sulphur from the third to sixth samplings. The plants from the check plot showed the highest percentage of sulphur throughout their entire growth, indicating that an ample supply of sulphur is present in the soil and that in the check plot sulphur took the place of some of the plant nutrients that were added to the fertilized plots.

This disposition of plants to take up readily available minerals is again demonstrated in the case of the completely fertilized plot 2. At four of the six sampling dates the plants from plot 2 had the lowest percentage of sulphur, showing that in the presence of an ample supply of phosphorus,

potassium, and nitrogen sulphur was used less extensively. Although the plants on plots 2 and 4 made nearly identical growth, the percentage of sulphur in the plants of plot 4 was at least 10 per cent. greater than that in the plants of plot 2 at all stages of growth.

The milligrams of sulphur per plant increased progressively in all plots during the entire growing season. Most of the sulphur, however, had been assimilated by the plant at the time of the second sampling. The percentages of the maxima at this time ran from 62.9 per cent. in the case of plot 6 to 81.7 per cent. in the case of plot 4.

No comparisons can be made with the English or the German investigations for neither study included a determination of sulphur. WOODFORD and MCCALLA (**16**) determined sulphur and found it increasing in the plants from the two plots grown on black soil until next to the last sampling, when a sharp decrease took place. The amounts of sulphur in the plants from the plots grown on the gray soil showed a slight increase from the first to the fourth samplings and remained practically constant from that time until harvest.

The data for sulphur apparently reflect the effect of the very dry weather and of the subsequent irrigation. The rate of assimilation diminished between the second and third samplings and increased again after water was finally applied to the plots.

NITROGEN

The graphical representation of the nitrogen data is given in figure 4. The percentage of nitrogen decreased asymptotically from the first to the last samplings. The percentages on the last four samplings are extremely close together. In fact on next to the last sampling date the percentage data from all six plots are very nearly within the limits of the experimental error of a Kjeldahl nitrogen determination.

These data demonstrate the effectiveness of a progressive development study in plant nutrition for it was only at the first two sampling dates that the effect of nitrates upon the percentage composition of the plant could be observed. At the first sampling the plants from plots 5 and 6, to which nitrate, and nitrate plus potassium were added, respectively, had the highest percentage of nitrogen and the next highest percentage was found in the plants of plot 2, fertilized with nitrate, potassium, and phosphorus.

When the amounts of nitrogen are plotted, wide differences become apparent between the various plots. The plants from plots 2 and 4 showed the highest amount of nitrogen at all sampling dates, with the plants from plot 2 running considerably above those from plot 4 at the third and fourth samplings. This may possibly be due to the fact that nitrate was added to plot 2 and hence was available during the dry period when the production of nitrates by soil bacteria had probably almost ceased.

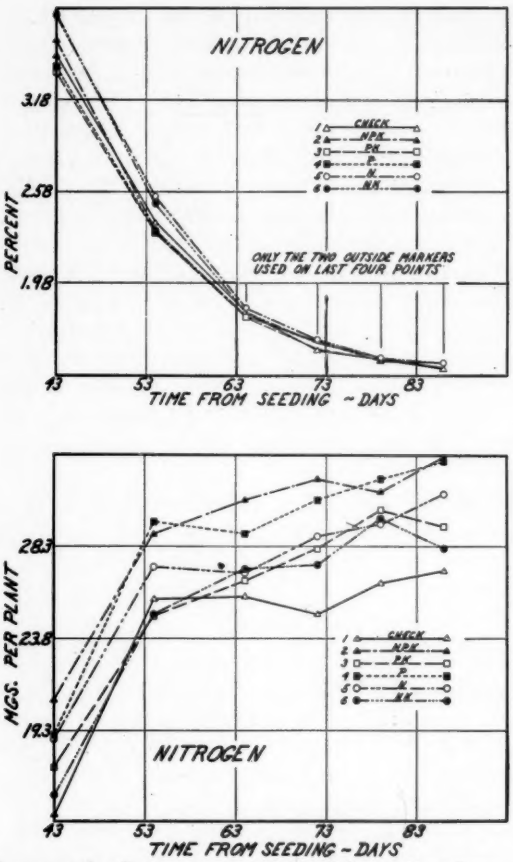

FIG. 4. Upper graph, nitrogen as percentage of dry matter; lower graph, nitrogen as milligrams per plant.

A large amount of nitrogen was assimilated by the wheat on all plots between the first and second samplings and a much smaller amount entered the plant from the second sampling until harvest. The amounts of nitrogen present at the second sampling as percentages of the maxima run from 82.7 to 94.8 per cent. However, the data show nitrogen increased until the final harvest on all plots except plots 3 and 6. These two plots showed a slight decrease between the fifth and sixth samplings.

The German investigation showed nitrogen reached a maximum three weeks before harvest and suffered a subsequent loss of 20 per cent. KNOWLES and WATKIN (9) found that nitrogen reached a maximum about four weeks

before harvest, but remained constant for the remainder of the growing season. BRENCHLEY (**4**) found assimilation of nitrogen proceeding until a week before maturity was reached. The investigation of WOODFORD and MCCALLA (**16**) shows nitrogen increasing until the final harvest.

CALCIUM

The data for calcium are presented graphically in figure 5. Little information can be drawn from the percentage data of the calcium determinations. The percentages of calcium in the plants from all the plots at the various dates of sampling are very similar. The percentage of cal-

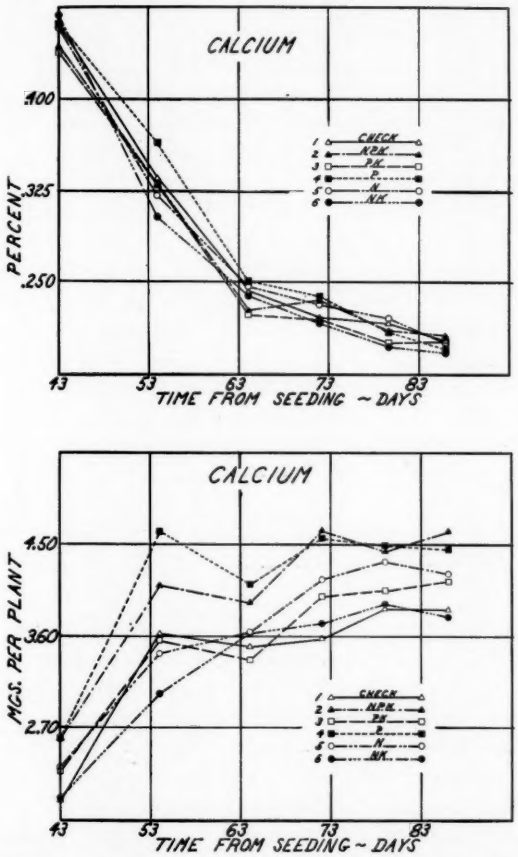

FIG. 5. Upper graph, calcium as percentage of dry matter; lower graph, calcium as milligrams per plant.

cium decreased in all cases from the first to the last sampling with by far the largest decrease taking place between the first and third samplings.

The data for the milligrams of calcium per plant show a heavy assimilation took place between the first and second samplings. With the exception of the plants from plots 5 and 6, there was a loss of calcium between the second and third samplings. Between the third and fourth samplings a reversal of the movement of calcium took place and there once more is a gain. From the fourth to the last samplings little change takes place. This reversal of movement of calcium was without doubt the effect of the hot dry weather upon the plant. When water was applied to the plants assimilation increased and there was a net gain in the amount of calcium in the plant.

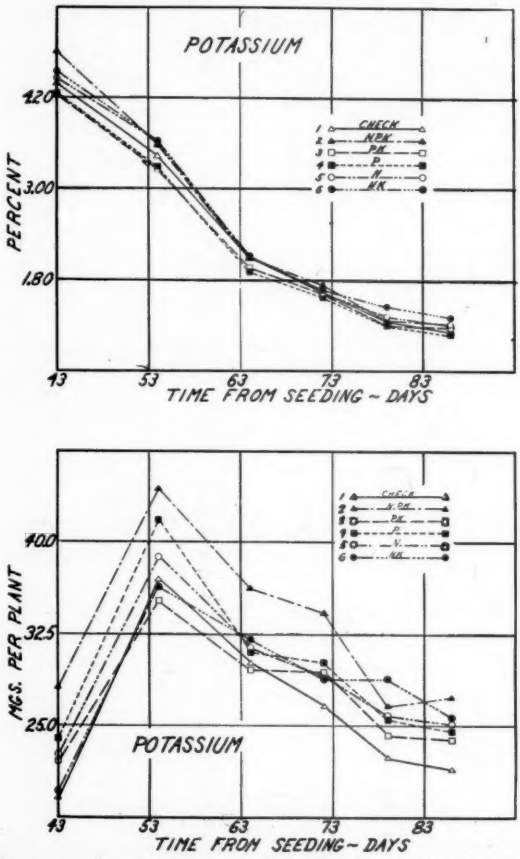

FIG. 6. Upper graph, potassium as percentage of dry matter; lower graph, potassium as milligrams per plant.

Calcium was not determined in the German investigation, but the English workers reported a loss of 19 per cent. of the calcium during the last six weeks of growth. The results of WOODFORD and MCCALLA (**16**) show a loss of calcium during the last fifteen days in the plants from two of their four plots. A small loss was noted in the present investigation in the case of the plants from plots 4, 5, and 6. The plants from plot 4 showed the largest amount of calcium at the second sampling, the plants from plots 1, 5, and 6 at the fifth sampling, and the plants from plot 3 at the last sampling.

POTASSIUM

The data for potassium are presented graphically in figure 6. The percentage of potassium in the plants from all plots decreased steadily from the first sampling until harvest with the plants from each plot showing a uniform decrease. The difference between the plants of the various plots was not pronounced but it will be noted that in all cases the plants from plots to which nitrate was added have the highest percentage of potassium.

The plants from plot 3, which was fertilized with phosphorus and potassium, and from plot 4, which was fertilized with phosphorus alone, had the lowest percentage of potassium at all stages of growth. The plants from plot 2, which received an application of nitrate in addition to the phosphorus and potassium, contained a higher percentage of potassium. These facts indicate that phosphorus depresses the assimilation of potassium unless there is an ample supply of nitrates available to the plant.

The total amount of potassium in the plants of all plots reached a maximum at the second sampling and decreased from that time until harvest. The amounts of potassium lost by the plants, expressed as percentages of the maxima, vary from 29.2 per cent. in the plants of plot 6 to 42.2 per cent. in the plants from plot 1. The rapid assimilation of potassium and the attainment of a maximum early in the growth of the wheat plant was observed by the German workers, the English workers, and WOODFORD and MCCALLA (**16**). BERRY (**3**) found the same thing in his study of the progressive development of the oat plant.

SOLUBLE ASH

The data for soluble ash are presented graphically in figure 7. The curves plotted for the percentage of soluble ash in the plants are nearly identical with the curves for the percentage of potassium in the plant. This is not strange when it is observed that the weights of potassium in the plants are nearly one-half the weights of soluble ash. When the weights of the anions accompanying the potassium are taken into consideration, it is evident that potassium salts must compose the major portion of the soluble ash.

The wheat from the three plots to which nitrates were added had the

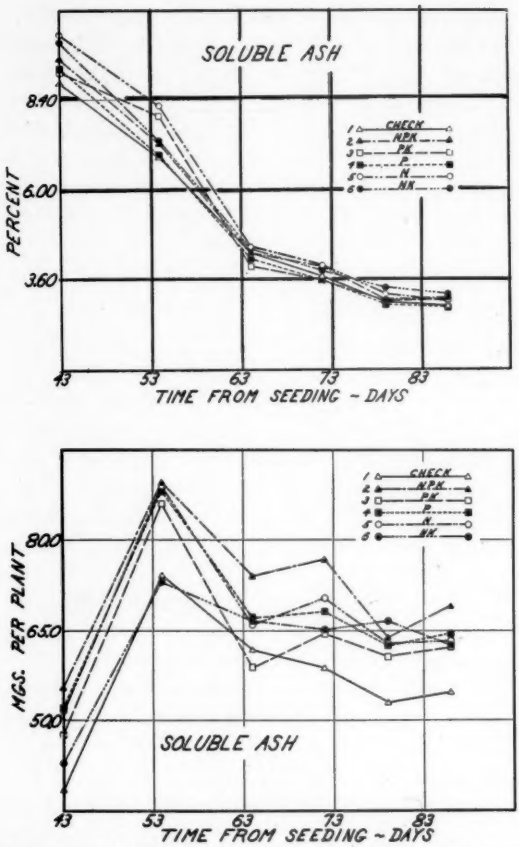

FIG. 7. Upper graph, soluble ash as percentage of dry matter; lower graph, soluble ash as milligrams per plant.

highest percentages of soluble ash. The wheat from these three plots also had the highest percentage of potassium.

The amount of soluble ash in the plants again reflects the behavior of potassium. Like potassium, the soluble ash reached a maximum in the plants from all the plots at the second sampling. From the second to the third sampling a considerable loss occurred, but in the interim between the third and fourth samplings the loss was not so large in the plants from plots 4, 5, and 6 and there was even a gain in the plants from plots 2, 3, and 4. This gain in soluble ash was probably due to the combined effect of the decrease in the downward movement of potassium and the increase in the assimilation of phosphorus, sulphur and calcium.

Ash

The data for ash are presented graphically in figure 8. The percentage of ash declined until the third sampling and then showed a slight increase

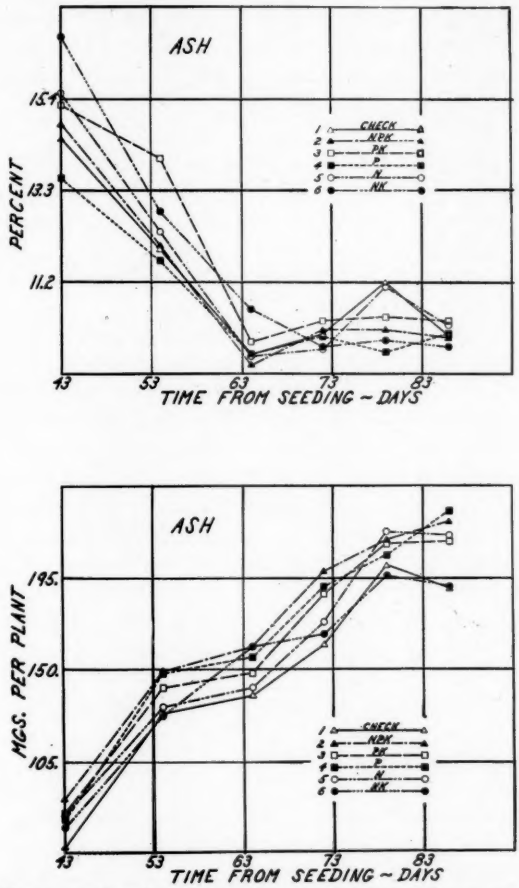

FIG. 8. Upper graph, ash as percentage of dry matter; lower graph, ash as milligrams per plant.

in most cases. An unusual jump in percentage of ash occurred in the plants from plots 2 and 5 at the fifth sampling date. The writer can advance no logical explanation for this unless it may be attributed to the error which would be reflected in the amount of ash by the inclusion of soil or dust particles in the samples. Care was taken to remove as much of the adhering

soil from the plants as possible, but it may be that some soil escaped detection. Any soil left adhering to the plants would, of course, add to the amount of ash determined.

The amounts of ash in the plants from all plots increased from the first to the fifth samplings. In the period between the last two samplings the amount of ash in the plants from plots 2, 3, 5, and 6 remained fairly constant while the ash in the plants from plot 4 continued to increase.

Effects of nutrients on the whole plant

The results have shown that the wheat grown for this investigation was still assimilating most of the plant nutrients at the time of harvest. Potassium reached a maximum at the second sampling date and suffered a subsequent loss, but sulphur, phosphorus, and nitrogen were still entering the plant when the last samples were collected. The amounts of calcium changed very little during the last two weeks of growth.

The previous studies on the progressive development of the wheat plant have shown assimilation ceasing earlier in the growth of the plant. The German workers (**15**) found nitrogen and phosphorus reached their maxima three weeks before harvest. Nitrogen suffered a subsequent loss and phosphorus remained constant. The English workers (**9**) found nitrogen and phosphorus assimilation ceased several weeks before harvest and the amount remained practically constant until harvest. They did not determine sulphur, but found calcium reached a maximum slightly in advance of phosphorus and nitrogen and later suffered a slight loss. Woodford and McCalla (**16**) found nitrogen increased until harvest. In three of their four plots phosphorus increased until harvest. Calcium showed but little change during the last three weeks. Sulphur reached a maximum two weeks before harvest and suffered a subsequent loss in two of their four plots. In the other two little change took place in the last three weeks.

The probable cause of this late assimilation of plant nutrients is the relatively short period of time which elapsed between seeding and harvesting of the wheat used in this study. The wheat was seeded on May 13 and harvested 87 days later on August 8. The average time between the seeding and harvesting of spring wheat in Montana is close to 120 days (**2**). The wheat used in the German study was seeded April 23 and harvested 127 days later on August 28. The period for the wheat used in the Canadian study was 114 days. It is apparent that an 87-day period is much shorter than the normal period of growth.

From the data of the three investigations in which spring wheat was grown, it is obvious that the length of the growing season may alter the conclusions reached in a study of this kind. The evidence indicates that the plant tends to assimilate nutrients over a certain length of time re-

gardless, or, perhaps we should say, in spite of its state of desiccation. Naturally the amount of moisture in a plant may in a large measure govern the movement of nutrients, but it is probably true that if the growing season of the wheat plant has been cut abnormally short by drought, nutrients will continue to be assimilated until late in the life of the plant.

As described earlier in this paper, the wheat was irrigated on July 21, three days before the fourth set of samples was collected. The effect of this irrigation upon the movement of nutrients in the wheat plant is definitely shown by the shape of the curves representing the milligrams of nutrients per plant. From July 6 to 16 there was a definite reduction in the rate of assimilation of nearly all the nutrients, indicating that the plants were drying out to such an extent that movement of nutrients was becoming extremely slow. The data for the last three samplings show the effect of the irrigation on July 21. It is evident that the roots of the plant were able to absorb enough moisture to allow the nutrients to once more resume their movement in the plant.

This is illustrated nicely by the movement out of the plant by potassium. Between the second and third samplings potassium moved out rapidly, but the rate decreased markedly between the third and fourth samplings. After the water was applied to the plots, the downward movement was again accelerated. Although the decrease in the movement of potassium was not found in the plants from plots 1, 5, and 6, it was very apparent in the plants from the remaining plots. It is interesting to note that in the case of all the nutrients determined, the added moisture resulting from the irrigation tended to produce an upward movement into the plant while in the case of potassium the downward movement was accelerated. Thus it appears that the downward movement of potassium is due in part to factors other than the natural desiccation of the plant as it approaches maturity.

The anions which are associated with potassium in its downward migration are not easily identified in this study. It is quite possible that some sulphate ions migrate from the upper portion of the plant with the potassium. As mentioned before, WOODFORD and MCCALLA (**16**) found a loss of sulphur occurred in the wheat from two of their plots. The English workers were the only ones to include a determination of chlorine and they found that chlorine moved downward in the plant at about the same time and at the same rate as did potassium. The loss in equivalents of chlorine, however, was far less than the loss in equivalents of potassium. It is possible, therefore, that the gain or loss noted in the nutrients of the wheat plant over a period of time is a net gain or loss resulting from the movement of ions both upward and downward in the plant.

The plants from plots 5 and 6, which were fertilized with nitrogen and nitrogen and potassium, respectively, exhibited a behavior decidedly

different from the plants of the other plots. This is especially true in the case of the plants of plot 6. It will be noted that the movement of most of the nutrients in the plants of these plots was less affected by the drought and subsequent irrigation. It has been frequently observed that an ample supply

TABLE VII

AMOUNTS OF SUBSTANCES IN LEAVES AND STEMS, AND IN HEADS, EXPRESSED AS MILLIGRAMS PER PLANT

PLOT	FERTILIZER TREATMENT	SUBSTANCES ON DATE OF SAMPLING					
		LEAVES AND STEMS			HEADS		
		JULY 24	JULY 31	AUG. 7	JULY 24	JULY 31	AUG. 7
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
		Dry matter					
1	Check	1130	1010	950	490	780	960
2	NPK	1320	1170	1180	650	940	1070
3	PK	1180	1080	1040	640	950	1020
4	P	1240	1180	1130	680	970	1150
5	N	1220	1150	1080	570	820	1030
6	NK	1130	1120	1000	590	870	970
		Phosphorus					
1	Check	1.06	0.61	0.47	1.76	2.53	3.22
2	NPK	1.19	0.69	0.55	2.31	2.81	3.36
3	PK	1.04	0.67	0.52	2.06	2.90	3.32
4	P	1.00	0.77	0.53	2.12	2.99	3.84
5	N	0.90	0.67	0.51	1.94	2.43	3.06
6	NK	0.88	0.65	0.45	2.03	2.57	2.77
		Sulphur					
1	Check	2.62	1.92	1.88	0.73	1.50	1.82
2	NPK	2.68	1.91	1.92	0.85	1.66	1.86
3	PK	2.54	1.87	1.78	0.87	1.60	1.87
4	P	2.68	2.22	2.06	0.96	1.68	2.04
5	N	2.64	2.09	2.01	0.82	1.38	1.94
6	NK	2.42	2.10	1.94	0.86	1.55	1.91
		Nitrogen					
1	Check	13.8	8.59	6.09	11.2	17.9	21.0
2	NPK	16.1	9.59	7.79	15.5	21.4	25.1
3	PK	13.7	9.07	6.66	14.5	21.0	22.6
4	P	14.8	10.3	7.12	15.8	21.3	25.5
5	N	15.3	10.9	7.67	13.5	18.5	23.2
6	NK	13.3	10.4	6.90	14.1	19.3	21.3
		Calcium					
1	Check	3.10	3.09	3.07	0.48	0.78	0.79
2	NPK	4.00	3.53	3.66	0.63	0.90	0.96
3	PK	3.35	3.20	3.28	0.64	0.85	0.86
4	P	3.86	3.65	3.48	0.70	0.84	0.97
5	N	3.57	3.58	3.33	0.59	0.75	0.88
6	NK	3.19	3.12	2.95	0.54	0.80	0.84

TABLE VII—(*Continued*)

Plot	Fertilizer treatment	Substances on date of sampling					
		Leaves and stems			Heads		
		July 24	July 31	Aug. 7	July 24	July 31	Aug. 7
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
		Potassium					
1	Check	22.3	16.2	14.3	4.32	6.10	7.09
2	NPK	28.2	18.8	19.2	6.03	7.76	8.08
3	PK	23.2	17.0	16.0	6.16	7.24	7.77
4	P	23.6	17.7	16.4	6.58	7.76	8.13
5	N	23.9	19.0	17.1	5.16	6.84	7.98
6	NK	23.3	20.8	17.8	5.50	8.02	7.92
		Soluble ash					
1	Check	46.3	34.5	32.8	12.6	18.6	21.9
2	NPK	60.2	41.4	45.1	16.6	22.5	24.1
3	PK	48.9	38.7	39.1	15.7	22.0	23.1
4	P	50.6	40.0	39.0	17.7	22.5	25.5
5	N	55.8	43.5	40.0	14.5	19.3	23.4
6	NK	49.9	45.1	40.0	15.3	21.6	22.6
		Ash					
1	Check	134	155	135	28.2	46.0	55.4
2	NPK	163	162	164	35.0	51.7	58.7
3	PK	152	158	155	34.9	54.1	57.5
4	P	153	152	161	38.0	53.7	66.6
5	N	142	171	159	31.1	47.4	57.3
6	NK	136	148	138	31.4	47.8	53.4

of nitrates produces heavy vegetative growth and tends to delay maturity (**13, 14**). The plants on these plots did not appear to have excessive leaf growth, but they did possess a high percentage of soluble salts. It may be that this fact contributed to their being less affected by the drought as regards the movement of nutrients.

Distribution of substances between heads and straw

The samples gathered on the last three sampling dates were divided into heads and straw for separate analyses. Table VII gives the amounts of substances, expressed as milligrams per plant, present in the heads and in the straw. Table VIII gives the amounts of substances present in the straw and in the heads, expressed as percentages of the dry matter, Table IX gives the amounts of substances present in the heads at the last three dates of sampling, expressed as percentages of the amounts present in the whole plant.

Figures 9 and 10 represent graphically the milligrams of substances present in the whole plant at the last three samplings and the distribution of these substances between the heads and the straw. The data from the three

successive samplings are grouped together for each plot. The six groups representing the six plots are arranged in numerical order from left to right. All eight charts in figures 9 and 10 are drawn to the same scale with the appropriate factor given with the name of the substance to illustrate the comparative amounts of the different substances present in the plant.

TABLE VIII

AVERAGE PERCENTAGE OF SUBSTANCES IN DRY MATTER OF LEAVES AND STEMS, AND OF HEADS

PLOT	FERTILIZER TREATMENT	SUBSTANCES IN DRY MATTER ON DATE OF SAMPLING					
		LEAVES AND STEMS			HEADS		
		JULY 24	JULY 31	AUG. 7	JULY 24	JULY 31	AUG. 7
		%	%	%	%	%	%
		Phosphorus					
1	Check	0.094	0.060	0.049	0.360	0.324	0.328
2	NPK	0.090	0.059	0.047	0.355	0.299	0.314
3	PK	0.088	0.062	0.050	0.322	0.305	0.325
4	P	0.081	0.065	0.047	0.312	0.308	0.334
5	N	0.074	0.058	0.047	0.340	0.296	0.297
6	NK	0.078	0.058	0.045	0.344	0.295	0.286
		Sulphur					
1	Check	0.232	0.190	0.198	0.148	0.192	0.190
2	NPK	0.203	0.163	0.163	0.131	0.177	0.174
3	PK	0.215	0.173	0.171	0.136	0.168	0.183
4	P	0.216	0.188	0.182	0.141	0.173	0.177
5	N	0.216	0.182	0.186	0.143	0.168	0.188
6	NK	0.214	0.188	0.194	0.145	0.178	0.197
		Nitrogen					
1	Check	1.22	0.85	0.64	2.29	2.30	2.19
2	NPK	1.22	0.82	0.66	2.39	2.28	2.35
3	PK	1.16	0.84	0.64	2.26	2.21	2.22
4	P	1.19	0.87	0.63	2.33	2.20	2.22
5	N	1.25	0.95	0.71	2.37	2.26	2.25
6	NK	1.18	0.93	0.69	2.39	2.22	2.20
		Calcium					
1	Check	0.275	0.306	0.323	0.097	0.092	0.082
2	NPK	0.303	0.298	0.310	0.097	0.100	0.090
3	PK	0.284	0.296	0.315	0.100	0.089	0.084
4	P	0.311	0.309	0.308	0.103	0.087	0.084
5	N	0.293	0.311	0.308	0.103	0.092	0.085
6	NK	0.282	0.279	0.295	0.092	0.092	0.087
		Potassium					
1	Check	1.97	1.60	1.50	0.882	0.782	0.739
2	NPK	2.14	1.61	1.63	0.927	0.825	0.755
3	PK	1.97	1.57	1.54	0.963	0.762	0.762
4	P	1.90	1.50	1.45	0.967	0.800	0.707
5	N	1.96	1.65	1.58	0.905	0.834	0.775
6	NK	2.06	1.86	1.78	0.933	0.922	0.817

TABLE VIII—(*Continued*)

Plot	Fertilizer treatment	Substances in dry matter on date of sampling					
		Leaves and stems			Heads		
		July 24	July 31	Aug. 7	July 24	July 31	Aug. 7
		%	%	%	%	%	%
		Soluble ash					
1	Check	4.10	3.42	3.45	2.58	2.38	2.28
2	NPK	4.56	3.54	3.82	2.56	2.39	2.25
3	PK	4.14	3.58	3.76	2.46	2.32	2.26
4	P	4.08	3.39	3.45	2.61	2.32	2.22
5	N	4.57	3.78	3.70	2.54	2.35	2.27
6	NK	4.42	4.03	4.00	2.60	2.48	2.33
		Ash					
1	Check	11.9	15.3	14.2	5.76	5.89	5.77
2	NPK	12.4	13.8	13.9	5.38	5.50	5.49
3	PK	12.9	14.7	14.9	5.46	5.69	5.64
4	P	12.3	12.9	14.3	5.59	5.54	5.79
5	N	11.7	14.8	14.7	5.46	5.78	5.56
6	NK	12.0	13.2	13.8	5.32	5.49	5.51

The percentages of substances present in the heads of the plants were in most cases quite different from the percentages of substances in the whole plant. It may be noted, however, that if a fertilizer treatment affected the

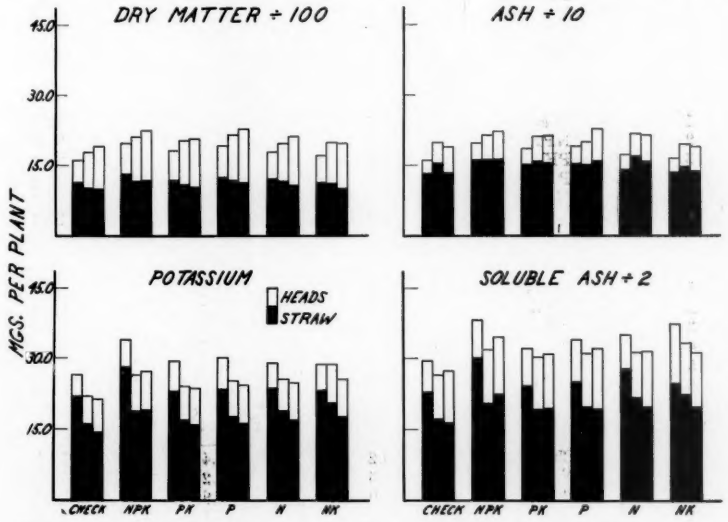

Fig. 9. Amounts of substances (expressed as milligrams per plant) present in the whole plant on the last three sampling dates and distribution of these substances between straw and heads.

TABLE IX

AVERAGE AMOUNTS OF SUBSTANCES IN HEADS OF PLANT EXPRESSED AS PERCENTAGES OF AMOUNTS PRESENT IN ENTIRE AERIAL PORTION

PLOT	FERTILIZER TREATMENT	AV. AMOUNTS OF SUBSTANCES ON DATE OF SAMPLING		
		JULY 24	JULY 31	AUGUST 7
		%	%	%
		Dry matter		
1	Check	30.2	43.6	50.3
2	NPK	33.0	44.5	47.6
3	PK	35.2	46.8	49.5
4	P	35.4	45.1	50.4
5	N	31.8	41.6	48.8
6	NK	34.3	43.7	49.2
		Phosphorus		
1	Check	62.4	80.6	87.3
2	NPK	66.0	80.3	85.9
3	PK	66.5	81.2	86.5
4	P	67.9	79.5	87.9
5	N	68.3	78.4	85.7
6	NK	69.8	79.8	86.0
		Sulphur		
1	Check	21.8	43.8	49.2
2	NPK	24.1	46.5	49.2
3	PK	25.5	46.1	51.2
4	P	26.4	43.1	49.8
5	N	23.7	39.8	49.1
6	NK	26.2	42.5	49.6
		Nitrogen		
1	Check	44.8	67.5	77.5
2	NPK	49.1	69.0	76.3
3	PK	51.4	69.8	77.1
4	P	51.6	67.4	78.2
5	N	46.9	62.9	75.1
6	NK	51.5	65.0	75.5
		Calcium		
1	Check	13.4	20.2	20.5
2	NPK	13.6	20.3	20.8
3	PK	16.0	21.0	20.8
4	P	15.4	18.7	21.8
5	N	14.2	17.3	20.9
6	NK	14.5	20.4	22.2
		Potassium		
1	Check	16.2	27.4	33.1
2	NPK	17.6	29.2	29.6
3	PK	21.0	29.9	32.6
4	P	21.8	30.4	33.2
5	N	17.7	26.5	31.8
6	NK	19.1	27.8	30.8

TABLE IX—(*Continued*)

Plot	Fertilizer treatment	Av. amounts of substances on date of sampling		
		July 24	July 31	August 7
		%	%	%
			Soluble ash	
1	Check	21.3	35.0	40.0
2	NPK	21.6	35.2	34.8
3	PK	24.3	36.2	37.1
4	P	25.9	36.0	39.5
5	N	20.6	30.7	36.9
6	NK	23.5	32.4	36.1
			Ash	
1	Check	17.4	22.9	29.2
2	NPK	17.7	24.2	26.3
3	PK	18.7	25.5	27.0
4	P	19.9	26.1	29.2
5	N	18.0	21.7	26.5
6	NK	18.8	24.4	28.0

composition of the whole plant in a certain manner, it also affected the composition of the head similarly. For example, the lowest percentage of sulphur was found in the plants from plot 2, and likewise the heads from plot 2 had the lowest percentage of sulphur.

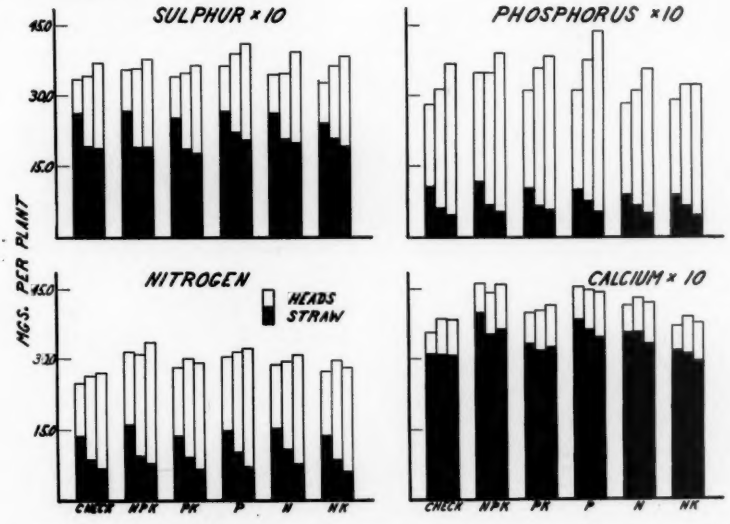

Fig. 10. Amounts of substances (expressed as milligrams per plant) present in the whole plant on the last three sampling dates and distribution of these substances between straw and heads.

The only exception found to this observation is the case of nitrogen. The percentage of nitrogen in the heads of plants from plot 2 was considerably higher than the percentage of nitrogen in the heads of the plants from the other plots in spite of the fact that the whole plants from all of the plots had nearly identical nitrogen percentages at the last three sampling dates.

At the last two sampling dates the straw of plots 5 and 6 had higher percentages of nitrogen than the straw from the remaining plots. The reason for this fact is seen in the values given in table IX, which show that a lower percentage of the nitrogen of the whole plant was transferred to the heads of the plants from plots 5 and 6.

There was remarkably little difference between the relative weights of heads per plant from the various plots. On all plots the heads formed almost exactly half the weight of the whole plant at harvest and it appears that the type of fertilizer applied to the plot had little effect upon this ratio.

Figures 9 and 10 show that there were approximately the same amounts of sulphur, phosphorus, and calcium present in the whole plant at maturity but that the distribution of these elements between the heads and the straw varied markedly. Approximately 85 per cent. of the phosphorus present in that portion of the plant lying above the ground was found in the heads. The heads contained approximately 50 per cent. of the sulphur and only 20 per cent. of the calcium. The elements nitrogen and potassium each represented about the same percentage of the dry matter of the mature plant. This percentage is approximately eight times the percentage of any of the three elements, sulphur, phosphorus, or calcium, and, althought nitrogen and potassium were present in the whole plant in approximately equal amounts, a large difference was found in the way they were distributed between the heads and the remainder of the plant. The heads contained about 75 per cent. of the nitrogen, but only 30 per cent. of the potassium. A greater proportion of phosphorus was found in the wheat heads than any of the five elements studied.

In nearly all cases the amounts of all the substances in the heads of the plants increased from the fourth to the sixth samplings. The rate of increase was very rapid between the fourth and fifth samplings and somewhat slower between the fifth and sixth samplings. Practically all the calcium and most of the potassium which entered the heads was found there at the fifth sampling. On the other hand, phosphorus, sulphur, and nitrogen were still entering the heads at the final sampling date.

Summary

1. The nutrition of Marquis wheat as influenced by five different fertilizer treatments was followed progressively through the growing season.

2. With few exceptions the amounts of dry matter, phosphorus, sulphur,

and nitrogen increased progressively from the first to the last sampling. Potassium reached a maximum in the plants from all plots one month before harvest and suffered a subsequent loss from that time until harvest. Calcium increased rapidly until one month before harvest and then remained fairly constant.

3. Curves for the rate of assimilation of the nutrients showed the effect of drought and subsequent irrigation upon the movement of plant nutrients. As the desiccation progressed there was a decreased movement of phosphorus, calcium, nitrogen, and sulphur into the plant and following the application of water the upward movement again increased. Potassium was moving out of the plant at this time and the desiccation of the plant decreased the downward movement. Following irrigation the downward movement increased.

4. The results show that the length of the growing season may alter the conclusions drawn from a progressive development study.

5. On all the plots the heads formed almost exactly half the dry weight of the aerial portion of the plant and the type of fertilizer treatment had little effect upon this ratio.

6. Approximately equal amounts of calcium, phosphorus, and sulphur were present in the plant at maturity, but the distribution of these elements between heads and straw varied markedly.

7. Practically all the calcium and most of the potassium which entered the heads was found there a week before harvest. Phosphorus, sulphur, and nitrogen, however, were still entering the heads when the grain was harvested.

DEPARTMENT OF CHEMISTRY
MONTANA AGRICULTURAL EXPERIMENT STATION
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SALT TOLERANCE OF PLANTS AT VARIOUS TEMPERATURES[1]

S. M. Ahi and W. L. Powers

(with three figures)

Introduction

The interesting observation was made that yellow sage (*Chrysothamnus viscidiflorus*) grows in Klamath Basin at some 4200 feet elevation on soil having a reaction of pH 9.5 to 10, while in the hotter climate of Malheur Valley, at an elevation of 2000 feet, it grows on soil of a reaction value of approximately 9. Small grain also appears to be more resistant to alkali at the cooler, more elevated alkaline areas. Eaton (**7**) has also suggested that temperature affects salt tolerance. Experiments were undertaken as a phase of a salt tolerance study to determine the effect of temperature on salt tolerance of certain plants, the degree to which certain plants differ in salt tolerance, the effect of chemical composition of the alkaline soils, and of other factors on salt tolerance.

Historical review

Reclamation and use of alkaline land is one of the major soil problems of arid agriculture. Harris (**16**) estimated that some 13 per cent. of the irrigated land contained sufficient alkali[2] to be seriously harmful to plants.

Evolution and classification of alkali soils

Any soluble salt that is present in the soil in excess may be called alkali. Hilgard (**22**) distinguished two types of alkali: (a) "white alkali" which includes one or more of such salts as sodium chloride, sodium sulphate, sodium nitrate, magnesium sulphate, magnesium chloride, calcium chloride; (b) "black" alkali, which usually consists mainly of sodium carbonate. "Black" alkali is more destructive to plants than "white" alkali.

Glinka (**12**) classifies soils into the three following groups: (a) solonchak, (b) solonetz, and (c) solod. According to Gedroiz (**8**) these three subgroups represent three consecutive stages of the process which may be called "evolution of alkali soil."

Such an evolution consists of three consecutive transformations: Salinization, which transforms ar original soil into solonchak; solonization, transforming solonchak into solonetz; and solotization, which transforms solonetz to the final product of the evolutionary process, solod (or soloth). Sol-

[1] Published as technical paper no. 275 with the approval of the Director as a contribution from the Soils Department of the Oregon Agricultural Experiment Station.

[2] "Alkali" land is here used as a popular term to refer to soil that is saline, or alkaline, or both.

onization, according to NIKIFOROFF (**27**), involves a desalinization with a partial substitution of monovalent exchangeable cations, especially sodium for calcium. Solotization involves hydrolysis and disintegration of the colloidal complex with a high degree of saturation of sodium, and a deflocculated state with soluble salts washed out and a columnar structure usually in the B horizon. The final product, solod, has reduced exchange capacity with the cations partly substituted by hydrogen ions, and the colloidal complex is partly destroyed, or reduced to its simple constituent oxides.

ALKALI INDICATORS

Native vegetation is a good indicator of the presence of alkali salts. HARRIS (**16**) states that there are at least 197 species native to California which are restricted to alkali soils. Some of these grow only when some particular salt is present, and others do well in the presence of a variety of alkali salts. In an arid region some plants may be found which indicate extremely large quantities of salts. Such plants are usually found alone. They indicate that so much alkali is present in the soil that agriculture without reclamation is impossible. According to HILGARD and others (**22**) crop plants may be grouped as follows:

(a) Sensitive—maize, wheat, young lucerne, bur clovers, peaches, oranges, prunes, etc.
(b) Resistant—oats, barley, sorghum, beets, etc.
(c) Very resistant—saltbush (*Atriplex confertifolia*), couch grass, date palms, etc.

Native vegetation, coupled with chemical analysis of soils make an excellent combination in determining the degree of contamination of saline land.

TOXIC LIMITS OF SALINITY

The amount of salt a given plant will tolerate depends upon the conditions under which it is grown. Some such tests have been confined directly to field observations, some to laboratory tests, and others to water culture experiments.

The limits of plant tolerance for sodium carbonate, sodium chloride, and sodium sulphate, as reported by HILGARD (**22**) are 0.1 to 0.25, 0.3 to 0.5, and 0.5 to 1 per cent. respectively. The results of HIBBARD (**21**) show that much smaller amounts may be very injurious in some cases, and larger amounts may cause little effect in other cases. HARRIS (**16**) states that more than 0.5 per cent. soluble salts where predominantly chloride, nitrates, and carbonates, and 1 per cent. where sulphates predominate were unsuitable for crop production without reclamation.

BANCROFT (**1**) found the following concentrations of salts killed bean plants:

Magnesium chloride	2640 p.p.m.
Sodium carbonate	2710 p.p.m.
Sodium nitrate	3700 p.p.m.
Sodium chloride	3600 p.p.m.
Sodium sulphate	6510 p.p.m.
Sodium bicarbonate	12,300 p.p.m.

His results indicate that magnesium chloride is the most toxic, and sodium bicarbonate is the least toxic. BREAZEALE (**2**) also places magnesium chloride as the most toxic among the salts occurring in alkali soil. He holds that toxicity is the function of both molecules and ions and that the toxicity may be determined by the units of time the plants have come in contact with alkali in that form during their long period of adaptation. He also states that the limit of endurance of a plant to an alkali is based on the composition of the soil solution at the wilting point of the plant and not upon the percentage of dry soil. This would necessitate giving the wilting points. On the other hand, KEARNEY (**24**) believes that salines can be classified only on the basis of percentages of salts referred to the dry weight of the soil. BUFFUM (**5**), working with wheat in Wyoming, demonstrated that salts prevent absorption of water by seeds so that germination is markedly retarded. A low concentration of salts may aid germination.

TOLERANCE OF VARIOUS CROPS FOR SALTS

Obviously it is impossible to indicate the precise tolerance of any plant, except for a given condition. The capacity of any plant to endure alkali according to HIBBARD (**18**) varies with physical character of soil, kind, amount, and character of alkali, fertility of soil, moisture supply, climate, kind of crop, and other modifying factors.

BREAZEALE (**3**) maintains that salt tolerance in plants is largely a matter of adaptation and development of physiological differences, such as development of mechanisms for lowering the rate of transpiration, or loss of water through the leaves. DORE (**6**) found higher suberin content in roots of salt-tolerant plants.

From a practical standpoint a good criterion of the tolerance of a plant for alkali, according to NEIDIG and MAGNUSON (**26**), is the capacity of that plant to grow to maturity and produce good yields, rather than its response during the germination period only. This may be of value for the specific purpose of investigation, but may be misleading otherwise. KEARNEY (**24**) places a critical concentration of white alkali for staple field crops at 1 per cent. and for most plants 0.3 per cent. He classifies salinity into seven grades, depending upon the percentage of salts.

HIBBARD (**20**) classes grasses among the most salt resistant cultivated plants, although he recognizes blue grass (*Poa pratensis*) and a few others

that are rather sensitive. Bermuda grass (*Cynodon dactylon*) and Rhodes grass (*Chloris gayana*) are especially tolerant to sodium sulphate.

ZOBELL and STEWART (29) have shown that asparagus grew well in soils containing 10,000 p.p.m. of sodium sulphate. Barley and oats appeared to be most tolerant cereals, while sweet clover was more tolerant than alfalfa, beans, or peas. As a rule forage crops have proved to be better adapted to alkaline soil than most cultivated crops. Quality of fruit, vegetables, sugar, and grain crops are more definitely impaired by alkali salts.

HILGARD (22) states that presence of salts in quantities as small as 200 to 300 p.p.m. is generally harmful to legumes. He places the limit for growth at about 1650 p.p.m. of total salts; at about 300 p.p.m. of sodium carbonate; and at about 1390 p.p.m. of sodium sulphate.

KEARNEY (24) places the highest amount for successful growth at 4000 p.p.m. of white alkali. Alfalfa, however, is very sensitive to sodium carbonate at the seedling stage. Old stands of alfalfa may tolerate some 2000 to 7000 p.p.m. of total salts, according to various authors, the lower limit being for sandy soil of low buffer value. With sodium carbonate the toxic limits varied between 300 and 900 p.p.m., where the salt was mostly sodium chloride. The variation in salt concentration designated by various authors is from 200 p.p.m. on sandy soil to 7100 p.p.m. on loam soil well supplied with moisture. Sweet clover seems to succeed better than alfalfa on alkali soils that are water logged or have shallow water table.

In explanation the literature on the subject of salt tolerance reveals that the studies may be grouped into two categories: (a) the amount of various salts added; and (b) the amount of salt recoverable in the water extract of the soil. Later studies indicate that only part of the added salts is recovered in water extract; hence the ratio of recovered to the total salts may not be the same as was revealed from salt additions.

HEADLY, CURTIS, and SCOFIELD (19) found that sodium carbonate added to the soil and allowed to remain several weeks is only partly recovered with the water extract of the soil. The limit of tolerance of crops to salts can best be determined by the amount of salt recovered from the soil rather than by the quantity added. The proportion of recoverable salts which killed half of the wheat seedlings was 0.04 per cent. for carbonates, 0.16 per cent. for chloride, and 0.35 per cent. for sulphate.

HARRIS and PITTMAN (18) have pointed out the discrepancy between the amount of added alkali salts and that recoverable in the water extract after such additions. They also found greater injury to plants grown in sand than to those grown in loam soil after each received the same amount of sodium carbonate.

TOLERANCE OF BACTERIA FOR SALTS

Effect of various salts upon the activity of ammonifying and nitrifying bacteria and other bacterial processes in the soil has been extensively investi-

gated. LIPMAN (**25**) reported sodium chloride toxic to ammonification by *B. subtilis*, and that the toxicity was reduced by the addition of magnesium or potassium chloride. Sodium carbonate was found stimulative in concentrations as high as 1 per cent., while 0.2 per cent. sodium sulphate reduced ammonification nearly one-half, and 0.2 per cent. sodium chloride reduced ammonification about two-thirds. BROWN and associates (**4**) found 0.06 per cent. calcium carbonate stimulating to ammonifiers, while 0.2 per cent. sodium carbonate, 0.1 per cent. sodium bicarbonate, and 0.5 per cent. sodium sulphate inhibited ammonification. GREAVES (**15**) found relative toxicity of various salts to nitrification decreased as the amount of moisture increased. GIBBS and associates (**10**) found toxicity of sodium carbonate to bacterial activities varied and was more tolerant toward the later period of growth. Change in chemical composition of protoplasm or in osmotic pressures produced may prevent bacteria from performing their normal functions, according to GREAVES, who found azofiers more resistant to alkali or neutral salts than ammonifiers, nitrifiers, and most higher plants. The common soil alkali, before retarding nitrogen fixation, then, would have to be present in quantities sufficient to greatly retard ammonification, nitrification, and plant growth.

Materials and methods

Experiments were undertaken to study temperature and other factors affecting the salt tolerance of salt grass (*Distichlis spicata*), alfalfa (*Medicago sativa*), sweet clover (*Melilotus* sp.), strawberry clover (*Trifolium fragiferum*), and a legume from Montana (*Astragalus rubyii*). Water culture, soil culture, and field plots were included.

Preliminary experiments were conducted with sea water, diluted to different concentrations with duplicate series of each concentration provided. The Pacific Ocean water after dilution was enriched by addition of Shive's culture solution of a strength equivalent to an osmotic concentration of one-tenth atmosphere. The reaction was adjusted and held at pH 5.7 to 6. Duplicate jars of "complete" nutrient solution equivalent to one atmosphere concentration were included as checks. In addition sodium chloride and sodium carbonate plus dilute culture solutions were employed. Two-gallon jars were used in all cases. Seeds were germinated in quartz sand and transplanted approximately when the fourth leaf appeared. One set of cultures was placed in a cold plant-house with a temperature of 55° F. and another was kept in a warm greenhouse at a temperature of 70°.

A constant temperature germinator built for Neubauer tests was made available for germination experiments under controlled conditions of temperature. Strawberry clover was germinated, and after eighteen days the percentage of germination was determined. Experiments were conducted

at 55, 70, and 90° F. The latter temperature proved so unfavorable that a second trial was made at a temperature of 85° F.

In addition a constant-flow experiment was conducted using sea water at various dilutions. The apparatus was adjusted to deliver a flow of some 2 liters per day.

Supplies of surface soil at the Vale Alkali Experiment Field were collected in June, 1936, from plats A, B, and N, and adjacent native hard alkali land previously described by JOHNSTON and POWERS (**23**).

Another lot sample of saline Yakima loam soil was obtained from near Klamath Falls. These soils were brought to the greenhouse, each lot mixed thoroughly and placed in duplicate series of one-gallon jars. Various chemical treatments were given to the soil to provide a series of pH values ranging from native alkali soil to neutrality. After planting, a favorable moisture content was maintained.

Chemical analyses of soil profile layers from the field tracts were made as to base exchange capacity, replaceable bases, water soluble salts, organic matter, available nutrients, and pH. Base exchange was determined by the barium chloride-ammonium chloride method, organic matter by Alexander's modifications of the Rather method, and pH with the hydrogen electrode. Certain biological activities of these alkali soils were studied. Detailed results, however, are to be reported separately.

Some moisture equivalent determinations were made using the centrifuge method.

The average salt content of sea water used for water culture experiments was 3.06 per cent. or 30,600 p.p.m. The dilutions used and dry weights of salt grass produced in the cold house and also in the warm house are shown in table I.

TABLE I

TOLERANCE OF SALT GRASS FOR SEA WATER

CONCENTRATION OF SEA WATER	WEIGHT OF DRY MATTER, AV. OF 2 CULTURES (FIRST CUTTING)		WEIGHT OF DRY MATTER, AV. OF 2 CULTURES (SECOND CUTTING)		TOTAL WEIGHT	
	COLD HOUSE	WARM HOUSE	COLD HOUSE	WARM HOUSE	COLD HOUSE	WARM HOUSE
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Complete nutrient solution	47.0	21.2	49.0	25.3	96.0	46.5
306 p.p.m. plus dilute nutrient solution	14.4	8.3	21.2	12.5	35.6	20.8
612 p.p.m. plus dilute nutrient solution	14.3	7.7	20.0	11.1	34.3	18.8
1834 p.p.m. plus dilute nutrient solution	12.7	7.7	13.1	9.5	25.8	17.2
2448 p.p.m. plus dilute nutrient solution	11.3	3.4	10.5	3.6	21.8	7.0

Experimentation

Water culture experiments

It is very interesting to note that in the cold house the total weight of dry matter obtained in the highest concentration of sea water is more than three times as much as that in the warm house. A significant difference in weight of dry matter of salt grass is shown in the various concentrations of sea water used (table I). The critical concentration of sea water for the conditions appears to be approximately 2448 p.p.m.

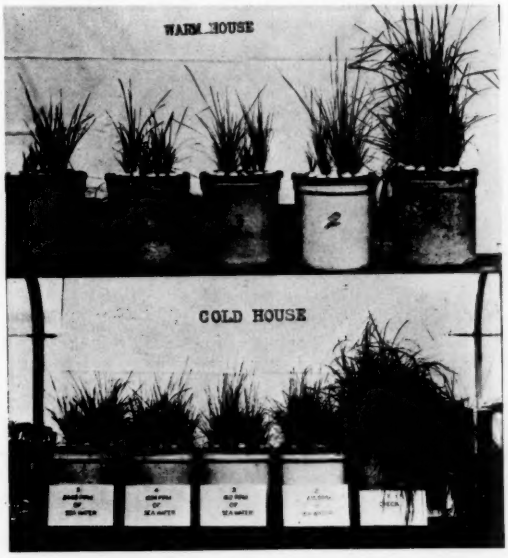

Fig. 1. Above, salt grass growing in various concentrations of sea water in warm house (about 75° F.). 1, check; 2, 306 p.p.m. of sea water; 3, 612 p.p.m. of sea water; 4, 1224 p.p.m. of sea water; 5, 2448 p.p.m. of sea water. Below, salt grass growing in the same concentrations of sea water in the cold house (about 55° F.).

This type of experiment was repeated with alfalfa using different concentrations of sodium chloride, sodium sulphate, and sodium carbonate as shown in tables II and III. The critical concentrations indicated by these tests are summarized in table IV. Combinations of different ratios of sodium carbonate to sodium sulphate indicate higher tolerance in combinations. These concentrations are lower than reported by Harris (**20**) for soil cultures. They are also lower than the concentrations of brackish water at high tide found bathing areas of salt grass growing on lowlands near the Pacific Coast in Oregon.

TABLE II

TOLERANCE OF ALFALFA FOR SEA WATER

CONCENTRATION OF SEA WATER	WEIGHT OF DRY MATTER, AV. OF 2 CULTURES (FIRST CUTTING)	WEIGHT OF DRY MATTER, AV. OF 2 CULTURES (SECOND CUTTING)	TOTAL WEIGHT
	gm.	*gm.*	*gm.*
Complete nutrient solution	13.4	18.3	31.7
350 p.p.m. plus dilute nutrient solution	13.1	16.1	29.2
700 p.p.m. plus dilute nutrient solution	13.8	11.3	25.1
1400 p.p.m. plus dilute nutrient solution	7.2	5.5	12.7

TABLE III

TOLERANCE OF ALFALFA FOR SODIUM CHLORIDE

CONCENTRATION OF SODIUM CHLORIDE SOLUTION	WEIGHT OF DRY MATTER, AV. OF 2 CULTURES (FIRST CUTTING)		WEIGHT OF DRY MATTER, AV. OF 2 CULTURES (SECOND CUTTING)		TOTAL WEIGHT	
	COLD HOUSE	WARM HOUSE	COLD HOUSE	WARM HOUSE	COLD HOUSE	WARM HOUSE
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Complete nutrient solution	15.0	13.0	15.8	13.6	30.8	26.6
350 p.p.m. plus dilute nutrient solution	13.5	11.9	14.7	11.1	28.2	23.0
700 p.p.m. plus dilute nutrient solution	11.8	9.4	13.4	10.6	25.2	20.0
1400 p.p.m. plus dilute nutrient solution	4.0	2.6	8.9	5.7	12.9	8.3

TABLE IV

CRITICAL SALT CONCENTRATIONS FOR CERTAIN PLANTS

PLANTS	CONCENTRATIONS			
	SEA WATER	SODIUM CHLORIDE	SODIUM SULPHATE	SODIUM CARBONATE
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
Salt Grass	10,000	1400	2800	700
Alfalfa	2800	1000	1400	350
Strawberry Clover	5600	.....	.....	.....

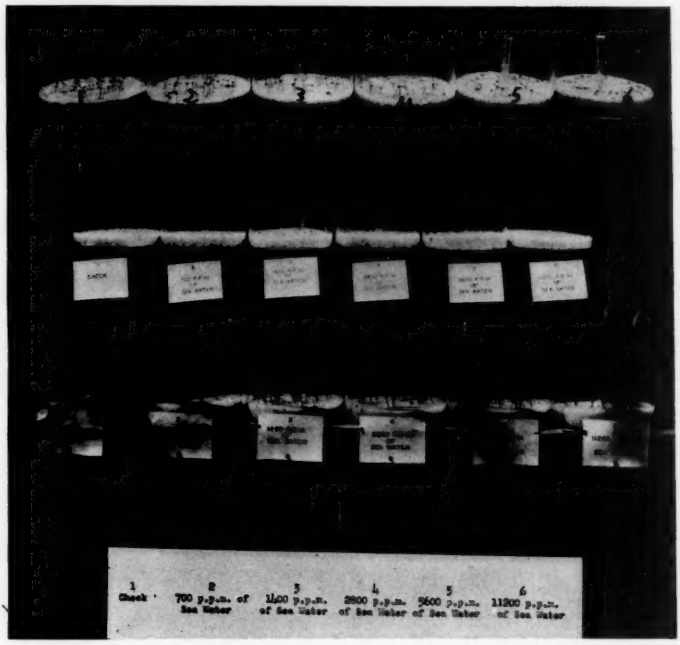

FIG. 2. Top row, strawberry clover grown at different concentrations of sea water increasing from left to right as indicated, at about 85° F. Middle row, same series of concentrations, germinated at 70° F. Bottom row, same series of concentrations, germinated at 55° F.

TABLE V

TOLERANCE OF STRAWBERRY CLOVER FOR SEA WATER AT CONSTANT CONCENTRATION

CONCENTRATION OF SEA WATER	WEIGHT OF DRY MATTER, AV. OF 3 CULTURES* (FIRST CUTTING)	WEIGHT OF DRY MATTER, AV. OF 3 CULTURES (SECOND CUTTING)	TOTAL WEIGHT
	gm.	*gm.*	*gm.*
Complete nutrient solution	4.0	6.50	10.50
700 p.p.m. plus dilute nutrient solution	2.67	2.95	5.62
1400 " " " " "	3.00	3.50	6.50
2800 " " " " "	1.00	2.10	3.10
5600 " " " " "	0.75	1.50	2.25
11200 " " " " "	0.50	0.50	1.00

* Only five seedlings were planted in each jar.

Strawberry clover is one of the more promising alkali-resistant legumes. It has been grown in recent field trials with fair success on Oregon soils with a pH up to 9 to 9.8 and also on acid soils with a pH value as acid as pH 4.2.[3] The tolerance of this plant for sea water in flowing solution cultures regulated to provide constant concentrations is shown in table V.

The critical concentration of salts which this plant may tolerate, under the conditions of the experiment, is indicated to be approximately 5600 p.p.m.

Sand culture experiments

From the better results secured in the cold house as compared to the warm house with a difference in mean temperature of approximately 15° F., it seemed desirable to further investigate this factor. A series of germination tests were, therefore, conducted using sand cultures in a germinator with temperature control. Strawberry clover and alfalfa seeds were used in these experiments. Lots of 100 seeds were germinated in clean quartz sand within large petri dishes for a period of 17 days. Water was added daily to maintain an even concentration and keep the cultures moist for germination. At the end of the period seedlings were counted and germination percentages calculated. The effect of temperature upon germination of seeds at various concentrations of sea water is shown in table VI. This

TABLE VI

Effect of temperature on germination of seeds at various concentrations of sea water

Concentration of sea water	Strawberry clover seeds germinated			Alfalfa seeds germinated		
	55° F.	70° F.	90° F.	55° F.	70° F.	90° F.
	%	%	%	%	%	%
No sea water	89.5	85.0	20.0	94.0	90.0	18.3
700 p.p.m. of sea water	95.0	87.0	5.0	93.0	82.2	3.0
1400 p.p.m. " " "	98.0	67.0	0.0	90.0	69.8	0.0
2800 p.p.m. " " "	85.3	79.0	0.0	90.0	38.5	0.0
5600 p.p.m. " " "	63.5	40.0	0.0	76.0	15.3	0.0
11200 p.p.m. " " "	47.7	0.0	0.0	38.0	0.0	0.0

effect is well illustrated in figure 2 where the relationship is shown more definitely than in the table by its effect on growth.

The effect of temperature is very striking. There is a definite decrease in the percentage of germination with increase in temperature or salt concentration. In high salt concentrations decrease occurs very rapidly at

[3] Tests of the tolerance of strawberry clover were made in cooperation with the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

FIG. 3. Growth of alfalfa on alkali land, check plat at left; reclaimed with sulphur at right, at Vale, Oregon, August, 1937.

high temperatures, but relatively slowly at low temperatures. At 90° F. there was practically no germination of seeds in the various treated cultures, while at 55° F. there was a high percentage of germination, except at the highest concentration. At the highest concentration there was no germination except with the lowest temperature which resulted in a germination of 47.7 per cent. for strawberry clover seeds, and of 38 per cent. for alfalfa. The two lower concentrations of sea water were stimulative to strawberry clover, but slightly retarded germination of alfalfa.

In comparing the two legumes, it will be noted that strawberry clover seeds germinate more satisfactorily than alfalfa in higher concentrations of sea water. In the presence of 2800 p.p.m. of total salts at ordinary growth temperature, strawberry clover germination in these tests was 78 per cent., as compared to 38.5 per cent. for alfalfa seeds. The strawberry clover is definitely more tolerant than the other seedlings tested and will germinate fairly well in the presence of as high as 5600 p.p.m. of total salts. Under cool and moist conditions, it may germinate in the presence of much higher concentrations.

SOIL CULTURE EXPERIMENTS

Series of soil culture experiments were initiated in the spring of 1936 using strawberry clover as indicator plants. Two soils were included in these investigations. One of these was an alkali soil brought from Vale Experiment Field and the other, a saline soil, was taken from near Klamath Falls. Both were loams. The initial reactions and chemical characteristics of these soils are presented in tables IX, X, and XI. Treatments were applied to give a reaction range or pH from nearly neutral to strongly alka-

line as shown in tables VII, VIII, and IX. The final soil reactions and results are also given in these tables.

TABLE VII

TOLERANCE OF STRAWBERRY CLOVER IN ALKALINE YAKIMA LOAM POT CULTURE

TREATMENT	WEIGHT OF DRY MATTER, AV. OF 2 CULTURES (FIRST CUTTING)	WEIGHT OF DRY MATTER, AV. OF 2 CULTURES (SECOND CUTTING)	TOTAL DRY WEIGHT	FINAL SOIL REACTION
	gm.	*gm.*	*gm.*	*pH*
No treatment	5.50	6.00	11.50	8.63
Sulphur, 1 T.*	6.00	7.00	13.00	7.61
Sulphur, 1 T.; manure, 20 T.	6.75	8.50	15.25	7.32
NaCl, 1000 p.p.m.	0.50	2.50	3.00	9.35
NaCl, 2000 p.p.m.	0.00	0.70	0.70	9.56
Na_2CO_3, 500 p.p.m.	0.00	1.20	1.20	9.48
Na_2CO_3, 1000 p.p.m.	0.00	0.40	0.40	9.78

* Tons per 2,000,000 lb. of soil.

Obviously the application of sulphur or sulphur with manure resulted in a greater yield of dry matter. This increase in dry matter was higher in the second cutting of the crop than in the first. The effect of sulphur with manure was more pronounced than that of sulphur alone.

The increase of dry matter due to the application of sulphur and sulphur with manure in the Yakima loam soil cultures was moderate while the effect was very marked in the Vale soil where it increased yields approximately threefold. The reactions induced would tend to reduce alkalinity but

TABLE VIII

TOLERANCE OF STRAWBERRY CLOVER IN LOAM SOIL FROM PLANT N, VALE EXPERIMENT FIELD

TREATMENT	WEIGHT OF DRY MATTER, AV. OF 2 CULTURES (FIRST CUTTING)	WEIGHT OF DRY MATTER, AV. OF 2 CULTURES (SECOND CUTTING)	TOTAL DRY WEIGHT	FINAL SOIL REACTION
	gm.	*gm.*	*gm.*	*pH*
No treatment	2.60	4.28	6.88	8.50
Sulphur, 1 T.*	5.10	6.75	11.85	7.39
Sulphur, 1 T.; manure, 20 T.	7.00	8.50	15.50	7.10
NaCl, 1000 p.p.m.	4.30	5.00	9.30	8.73
Na_2CO_3, 500 p.p.m.	3.10	2.50	5.60	8.82

* Tons per 2,000,000 lb. of soil.

TABLE IX

RESPONSE OF VARIOUS LEGUMES IN ALKALINE SOILS WITH OR WITHOUT TREATMENTS

SOIL	TREATMENT	DRY WEIGHT OF ALFALFA, AV. OF 2 CULTURES	DRY WEIGHT OF STRAWBERRY CLOVER, AV. OF 2 CULTURES	DRY WEIGHT OF SWEET CLOVER, AV. OF 2 CULTURES	DRY WEIGHT OF *Astragalus rubyii*, AV. OF 2 CULTURES
		gm.	*gm.*	*gm.*	*gm.*
Virgin alkaline soil, Vale Experiment Field	No treatment	0.25	2.25	1.40	0.0
	Sand (200%)	3.35	3.98	3.70	0.0
	Gypsum (12 T.)*	5.20	5.05	5.50	0.0
	Sulphur (2 T.)	5.50	5.87	6.95	0.0
	Sulphur (2 T.) Manure (20 T.)	7.90	8.86	12.90	4.1
Plat A Vale Experiment Field	No treatment	6.00	6.70	6.35	0.0
	Sand (200%)	6.30	7.15	6.20	0.0
	Gypsum (12 T.)	6.40	8.15	6.90	0.0
	Sulphur (2 T.)	6.70	8.10	6.40	1.25
	Sulphur (2 T.) Manure (20 T.)	6.75	8.90	7.70	5.2
Plat B Vale Experiment Field	No treatment	2.90	3.20	2.10	0.0
	Sand (200%)	3.30	3.67	3.15	0.0
	Gypsum (12 T.)	4.95	5.00	3.40	0.0
	Sulphur (2 T.)	5.00	5.17	3.70	0.0
	Sulphur (2 T.) Manure (20 T.)	5.50	5.90	4.20	4.6

* Tons per 2,000,000 lb. of soil.

might increase total soluble salts. More growth was secured on untreated Yakima loam than on the check jars of more alkaline Vale loam, which was in a poor physical condition.

Sodium chloride and sodium carbonate treatments were detrimental to the growth of plants in both soils, although 1000 p.p.m. of sodium chloride did not decrease the total yield of dry matter on Vale soils.

From the data, it may be concluded that strawberry clover will make a better growth on alkaline, or saline soils, provided the soil is well supplied with sulphur and manure accompanied by leaching. Sulphur oxidizes and brings calcium into solution to replace exchangeable sodium. One effect of manure is to supply microorgansms for biological oxidation of the sulphur.

CHEMICAL ANALYSES OF SOILS

The soils that were used in the greenhouse were sampled in the field by horizons, 0 to 5, 5 to 20, and 20 to 40 inches depth. Determinations of replaceable bases, soluble salts, reaction, total nitrogen, and organic matter, as

TABLE X

REPLACEABLE BASES IN VALE ALKALI EXPERIMENT PLATS. SAMPLED IN FALL, 1936

PLAT	TREATMENT	DEPTH	BASE EXCHANGE CAPACITY PER 100 GM.	EXCHANGE Ca – Mg PER 100 GM.	EXCHANGE Na PER 100 GM.	EXCHANGE K PER 100 GM.	Ca AS PART OF EXCHANGE CAPACITY	Na AS PART OF EXCHANGE CAPACITY
		inches	*M.E.*	*M.E.*	*M.E.*	*M.E.*	%	%
		0 – 5	24.25	2.48	20.26	1.51	10.22	83.13
Virgin alkali soil		5 – 20	28.60	0.65	26.76	1.19	2.27	93.56
		20 – 40	22.61	2.05	19.50	0.96	9.06	86.68
	Manure (10 T.)	0 – 5	20.75	15.95	4.10	0.70	76.87	19.76
	6 yrs. (60 T.)	5 – 20	22.26	16.84	4.72	0.70	75.65	21.20
Plat A	Sulphur (1.5 T.)	20 – 40	24.17	20.78	3.02	0.39	85.90	12.49
		0 – 5	27.49	4.75	21.09	1.65	17.28	76.72
		5 – 20	32.34	5.93	24.15	2.26	18.34	77.77
Plat B	Untreated	20 – 40	33.34	12.84	18.19	1.31	39.69	56.25
		0 – 5	12.94	9.78	2.73	0.43	75.50	21.10
		5 – 20	18.33	13.74	3.14	1.45	74.90	17.10
Plat N*	Sulphur (500 lb.)	20 – 40	24.02	21.00	2.90	0.16	87.50	12.50
Yakima loam†		0 – 6	19.94	5.80	12.51	1.63	29.10	62.74
from Klamath Falls		6 – 18	17.79	8.60	8.05	1.14	48.31	45.23
		18 – 28	17.25	8.33	7.93	0.94	48.28	46.51

* Sampled in April, 1936.
† Sampled in June, 1936.

TABLE XI

WATER-SOLUBLE SALTS IN VALE ALKALI EXPERIMENT PLATS. SAMPLED FALL, 1936

PLAT	DEPTH	TOTAL SOLUBLE SALTS	CARBONATE (CO_3)	BICARBONATE ($H\,CO_3$)	SULPHATE (SO_4)	CHLORIDE (CL)	SODIUM (NA)
	inches	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Virgin	0 – 5	3150	1019	.....	78	108	1200
alkali	5 – 20	4700	811	.....	1197	427	1509
soil	20 – 40	3480	811	.....	607	230	1345
	0 – 5	560	0	82	78	55	396
	5 – 20	960	0	82	66	56	387
Plat A	20 – 40	880	0	41	102	47	453
	0 – 5	2950	811	1237	210	95	1057
	5 – 20	3500	761	1016	535	273	1369
Plat B	20 – 40	2020	406	412	197	148	2121
	0 – 5	1310	0	330	82	76	969
	5 – 20	930	0	491	87	52	1465
Plat N*	20 – 40	1320	0	742	107	57	280
Yakima loam†	0 – 6	4010	0	2474	119	109	1294
from	6 – 18	1980	Trace	1640	99	58	948
Klamath Falls	18 – 28	1710	Trace	1237	99	57	671

* Sampled in April, 1936.
† Sampled in June, 1936.

well as moisture equivalent were made. In addition, the relationship of exchangeable sodium and exchangeable calcium were calculated and reported. The object of this investigation was to determine the extent of alkalinity or progress of reclamation and also to correlate the results with the general growth of plants on such soil.

REPLACEABLE BASES.—The amount and kind of adsorbed bases is one of the important properties indicating the extent of alkalinity or progress of reclamation. Table X shows the content of replaceable bases and the proportion of univalent cations (Na) and divalent cations (Ca – Mg) to the total exchange in all soil profiles. Plat A which was treated with sulphur and manure is now largely saturated with calcium, while plat B is still mostly saturated with sodium. The proportion of divalent cations in the 40-inch profile is much higher in plat A than in plat B. The replaceable sodium shows a reverse relationship indicating little modification in plat B from virgin soil. The exchange of calcium for sodium is most nearly complete in plat A which receives most sulphur and manure. A similar relationship exists in plat N indicating that alkaline soils could be effectively reclaimed. The treated plats show good exchange capacities, although

lower than those of virgin alkali samples. The high content of replaceable sodium in virgin soils from such plats will explain the high alkalinity of the soil. The Yakima loam soil from Klamath Falls contains replaceable sodium as well as salts to account for the unproductivity of the soil.

WATER SOLUBLE SALTS.—The soluble salts in the various horizons of different profiles are shown in table XI. There is marked difference between the check plat and the treated plat. The check plat contains more than five times as much water-soluble salts in the surface layer as that in plat A. There is slight difference in the total salt content between the check plat and virgin soil. Plat N, although the salt content is much lower as compared with the check plat, contains sufficient sodium salts to be a factor in the abnormality of the soil. In both treated plats the alkaline carbonate has disappeared throughout the whole profile studied. There is little change in alkaline carbonate in the check plat as compared to the virgin soil. Sulphates and chlorides are markedly diminished in the treated plats. In most every case both sulphates and chlorides are concentrated in the 5- to 20-inch layer of the soil profile. The relative amount of soluble sodium removed from the check plat has been very slight. Only with chemical treatment is there a marked elimination of soluble sodium. The Yakima loam soil shows a higher salt content than the treated land from Vale.

The salt content of the surface layer is even higher than that of the virgin alkaline soil. It appears that the Yakima loam soil is of a saline type as compared with the alkaline soils from Vale.

AVAILABLE NUTRIENTS.—The available nutrients in saline or alkaline soils are almost invariably low and the reaction is too alkaline for best growth of crop plants in many cases. The low content of nitrogen is the natural result of sparse vegetative cover which the soil supports. The effect of treatment on available nutrients is well illustrated in table XII.

It will be noticed that the reaction of the soil of plat A is approaching neutrality. The reaction of the two treated plats in general is well within the growth range for alfalfa. There is a relative increase in total nitrogen in treated plats as compared with that of the checks as reported earlier by POWERS (**28**). The increase in total nitrogen is due to a corresponding increase in organic matter. The soluble phosphorus is higher in the virgin soils than the other plants. This is perhaps due to the phosphorus being in the form of sodium phosphate, and also the plats may have lost some phosphates. The effect of treatment is further indicated by the amount of soluble calcium. In all layers the treated plats contain approximately twice as much soluble calcium as that of the checks. This shows that there is a tendency for the added material to bring calcium into solution.

The effect of reclamation is well demonstrated in figure 3. This relationship is more apparent in table XIII where the yields of hay per acre

TABLE XII

AVAILABLE NUTRIENTS IN VALE ALKALI EXPERIMENT PLATS. SAMPLED FALL, 1936

PLAT	DEPTH	REACTION	TOTAL NITROGEN	ORGANIC MATTER	WATER SOLUBLE PHOSPHORUS AS PO_4	WATER SOLUBLE CALCIUM	MOISTURE EQUIVALENT
	inches	*pH*	%	%	*p.p.m.*	*p.p.m.*	%
Virgin	0 – 5	9.87	0.054	1.31	13.9	76	29.3
alkali	5 – 20	9.89	0.028	1.17	32.3	82	27.3
soils	20 – 40	9.80	0.024	1.02	27.8	82	30.1
	0 – 5	7.60	0.093	2.39	6.1	122	34.1
Plat A	5 – 20	8.50	0.056	1.32	5.0	90	37.1
	20 – 40	8.77	0.028	0.91	16.1	111	35.4
	0 – 5	9.40	0.046	1.08	18.9	56	26.9
Plat B	5 – 20	9.92	0.044	1.08	1.1	68	33.7
	20 – 40	9.32	0.028	1.01	3.0	62	36.1
	0 – 5	8.75	0.123	1.34	10.4	125	.....
Plat N*	5 – 20	8.15	0.079	1.46	3.0	105	.....
	20 – 40	8.18	0.082	1.25	8.9	119	.....
Yakima loam†	0 – 6	9.45	0.107	2.48	8.9	121	.....
from	6 – 18	9.10	0.076	1.70	18.9	68	.....
Klamath Falls	18 – 23	9.05	0.044	0.82	12.3	64	.....

* Sampled in April, 1936.
† Sampled in June, 1936.

TABLE XIII

YIELDS OF ALFALFA HAY SECURED ON VALE ALKALI EXPERIMENT PLATS FROM 1931 TO 1936

PLAT	TREATMENT	POUNDS PER ACRE					
		YEAR					
		1931	1932	1933	1934	1935	1936
		lb.	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*
A	Manure (10 T.) 6 yrs. (60 T.) Sulphur (1.5 T.)	3720	3180	6480	7112	4902	4860
B	Untreated	0	220	680	610	780	1256
C	Manure (8 T.) 5 yrs. (40 T.) Sulphur (1500 lb.)	2020	4600	3060	3220	3790	5292

obtained on plats A and B for a period of years is presented. It will be noted that in each the yield obtained in plat A is much higher than that obtained from plat B. Although there is some seasonal variation, in part due to water shortage and injury to the stand by freezing, still the treated plat has produced rather good yields for the locality. General indications are very promising as to the ultimate success of reclamation. In these plats, alfalfa tends to become chlorotic when the reaction value of pH 8.6 or higher is obtained. Sulphur and manure, when used together, have been very effective in the reclamation of these alkaline soils.

Where strawberry clover seedlings were chlorotic the soil tested pH 9.2, and where color of seedlings was normal it tested 9 early in the season. In September chlorotic plants were found on soil that had a pH of 10. At this time plants of good color were found to be maturing seed on nearby soil having a pH value of 9.71. These tests were with samples obtained near Klamath at 4100 feet elevation.

Samples taken from soils near Vale at 2200 feet elevation June 10, where strawberry clover seedlings had good color, showed a pH value of 8.8; and where seedlings were chlorotic, the soil pH was 9.3. In September strawberry clover plants appeared thrifty at a pH value of 8.83, while a soil on which chlorotic plants were growing had a pH value of 9.15. A seed yield of 300 pounds per acre was obtained near Vale.

Near Baker, at 3400 feet elevation, strawberry clover in September, 1937, appeared thrifty with soil pH of 9.45 and chlorotic with soil pH of 9.99.

Prosser Station, Washington, reports (mimeo 17th annual field data July 9, 1937): "the average concentration of salts in soils that strawberry clover plants will tolerate is 0.4 per cent."

Microbial studies[4]

Studies of the effect of alkali salts on the activities of desirable soil microorganisms will be treated only briefly. A poor physical condition of alkali soils may check the activity of such organisms, and the concentration of salts may result in plasmolysis. Data from table XII show an improvement in moisture equivalent, indicating improved structure, water capacity, and perhaps carbon dioxide evolution resulting from the partial reclamation of certain plats, such as plat A.

Various materials were applied to these alkali soils and observations made as to the carbon dioxide evolved as well as the change in number of bacteria and molds. In general, the various treatments were effective in increasing the number of microorganisms.

There was a significant difference in the sulphur-oxidizing power of the soil. At the close of the experiment 100 per cent. of the sulphur added in the reclaimed soil was oxidized while only 20 to 30 per cent. of the sulphur was oxidized in the virgin soil. Sulphur oxidation in both soils was most effective in the first 115-day period as compared with the second interval of 100 days.

This was not true so far as *Azotobacter* is concerned. No development of these organisms occurred during the first period while in the second period moderately heavy development occurred. In general, the various treatments were effective in increasing the number of microorganisms. Similar results were obtained in the reclaimed land with the exception that in most cases moderate development of *Azotobacter* occurred in the second period as compared to the heavy development in the first period. The treatment increased the amount of water-soluble phosphorus, especially where sulphur was applied.

Discussion

From results obtained with salt-tolerance studies, temperature and other factors have been shown to be of vital importance. Results should be of practical application in selection of soils or providing conditions favorable for plant growth.

Nature of injury

Soluble salts may cause injury to plants by preventing them from absorbing moisture; by direct corrosive action; by toxicity due to excess of salt or its ions, or the products of hydrolysis; by inhibiting biological activities; by their effect upon germination; or by producing an abnormal physical condition in the soil. Seeds may germinate in saline soil and the young seedlings may be killed by the high and changing concentration of the soil

[4] Dr. W. B. Bollen, of the Oregon State College, Department of Bacteriology, supervised the microbial studies reported herein.

solution. In general older plants are more resistant than young seedlings. Plants growing on alkaline soil have characteristics similar to those found under desert conditions. They lack the bright green appearance of vigorous, healthy plants.

Under the conditions of the experiment the concentration of sodium carbonate which the salt grass may tolerate is 700 p.p.m. It may be possible that under field conditions the mature plants would do well in higher concentrations than results here would indicate. The limit of tolerance of salt grass has been found to be 1400 p.p.m. for sodium carbonate and 2800 p.p.m. for sodium sulphate.

Alfalfa, although sensitive to salinity at the seedling stage, made a fairly good growth in various concentrations of salt with indications of better growth under cooler conditions.

Results of sand culture tests clearly indicate (a) that strawberry clover is superior under alkaline or saline conditions to alfalfa, and (b) that temperature plays an important part in the germination and growth of plants in saline soils. Further evidences are found from concentration experiments using sea water, as clover, on the soil used, is found to tolerate a concentration as high as 5600 p.p.m. of total salts. The results are affected by temperature for the germination is not so good with more heat. It seems sufficient here to present conclusive evidence that differences in salt tolerance occur with changes in temperature. Water-culture experiments with salt grass and alfalfa present further confirmatory evidence that temperature is an important factor in the germination and growth of plants under saline conditions. The following suggestions are offered to the effect that temperature may be due to the action of one or the combined action of three or more factors. In the first place, it may be attributed to the "heat" factor only, since there is a reduction in the seed germination even in the check. This factor may be active only at a temperature of 90° F. because at 73° F., which is the normal growth temperature, no detrimental effect is noted due to the heat. Since the reduction of approximately 80 per cent. germination occurred in the checks at 90° F. it seems safe to state that heat is a factor.

In the second place, the relationship of temperature to germination may be due to the function of both the molecules and the ions. Previous evidence by BREAZEALE (**2**) supports the above statement. It is well understood that rise in temperature will increase the activity of ions due to the high degree of dissociation of the salt. The sea water is largely composed of easily ionizable salts and an increase of 20° F. should increase the number of ions present. This greater concentration of ions may cause greater toxicity; hence the complete retardation of germination.

Finally, the prevention of germination at 90° F. may be due to the rate

of mobility or diffusion of the salt. An increase in temperature results in an increase in the rate of diffusion which may ultimately cause the complete inhibition of germination. Possibly the increased activity of ions may be the only logical explanation of the temperature effect upon the germination of seed. Fertilizer burning in hot weather is closely related to salt tolerance. Tolerance will vary with age and kind of crop.

The results of chemical analyses and those of salt tolerance studies are not in full agreement. Improved chemical conditions in the soil will increase yields in the field. It was not fully apparent under greenhouse conditions, but this may be due to the following factors:

(a) The physical abnormality of the soil. When a comparative trial was made on the same soil diluted with sand the crop made a better growth.

(b) The lack of desirable microorganisms or their activities in the soil. This is indicated by the best growth of crops in pots treated with sulphur and manure, wherein the manure may have harbored microorganisms that speed up desirable microbiological activities.

(c) Replaceable sodium. Even in the better plats replaceable sodium is still present in moderate amounts.

(d) Under greenhouse conditions soil, light, relative humidity, and other factors are modified.

The virgin alkali plat soil was found to harbor an active and versatile microbial population. There was a significant difference, however, in the oxidizing power of the soils. The sulphur-oxidizing power of reclaimed soil is much greater than in virgin land. Salts applied to the Willamette silty clay loams in western Oregon had a depressing effect upon microbial activity in the soil. All of the salts used showed an inhibiting effect upon numbers and activities of microorganisms, sodium chlorate being the most effective.

Summary

1. Temperature is a dominant factor in the germination and growth of plants under saline or alkaline conditions. Temperature relations should be considered before recommending certain crops for saline soils.

2. Strawberry clover was found the most promising resistant legume for salinity, followed by sweet clover, then alfalfa. *Astragalus rubyii* failed to grow well under different treatments, with the exception of the soil receiving sulphur and manure.

3. Chemical analyses show a marked improvement of treated plats over those of checks by restoration of calcium for sodium in the exchange complex, improved reaction, decreased salt concentration, higher level of organic matter and nitrogen, a tendency to increase base exchange capacity, and useful water capacity or moisture equivalent. The crop yields obtained under field conditions substantiate the chemical results.

4. The sulphur oxidizing power of reclaimed soil was found to be much greater than that of the virgin alkali soil. Various treatments increased nitrate nitrogen.

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TRANSLOCATION IN PLANTS

A. S. Crafts

Introduction

Elimination of certain untenable theories has narrowed recent research on translocation to a search for the mechanism of rapid longitudinal movement of foods through phloem. (For comprehensive bibliographies see **22, 48,** and **51.**) Present theories fall into two categories: (1) movement of solute molecules taking place in, through, or upon the surface of sieve-tube protoplasm, and which results from protoplasmic activity; (2) mass flow of solution through sieve tubes or phloem, related, at least indirectly, to activity of photosynthetic tissues, and not dependent upon the activity of the sieve-tube protoplasm.[1] Both mechanisms are based upon ringing experiments which demonstrate limitation of primary movement of foods to the phloem, and upon analytical data which indicate concentration gradients between regions of synthesis and utilization.

Evidence for the protoplasmic streaming hypothesis has been reviewed by Curtis (**22**; also **9** and **25**); and Mason and Phillis have recently elaborated an activated diffusion theory (**45, 46, 47, 48**) to explain solute movement via the sieve-tube protoplasm. These workers postulate an independent movement of various solutes resulting from independent gradients; they call upon vital activities or surface phenomena to explain the acceleration required.

This paper presents the case for mass flow, drawing attention to certain quantitative evidence and to cytological studies on the activity of sieve-tube protoplasm.

Concentration gradients of organic solutes in the phloem have been adequately demonstrated.[2] That they cause an effective diffusional movement of the solutes is less certain. Physical aspects indicate that diffusion, over long distances, is naturally a slow process; and acceleration of unilateral solute movement, to the required rates, independent of the solvent, would necessitate an immense expenditure of energy. Mason and Phillis (**48**) have recognized this fact.

The mass-flow hypothesis involves a mechanism for transforming concentration gradients into pressure differences. The essential features of this mechanism are apparently incorporated in the normal structure of plants (**11, 50**). Exudation studies (**12, 15, 50, 51**) indicate a maintained

[1] Huber has recently proposed a somewhat similar classification (Huber, Bruno. Hundert Jahre Siebröhren-Forschung. Protoplasma **29**: 132–143. 1937.).

[2] See also Huber, B., Schmidt, E., and Jahnel, H. Untersuchungen über den Assimilatstrom. I. Tharandt Förstl. Jahrb. **88**: 1017–1050. 1937.

pressure gradient in the phloem; and studies on virus movement by BENNETT (**2, 3, 4**) and others, which indicate a marked parallelism between translocation of virus and organic foods, cast doubt on the independent movement of solvent and solutes. Those who favor mass-flow question the evidence for independent movement of various solutes; and they present anatomical, physiological, and cytological data which indicate (1) that the total phloem (or sieve-tube strand) is the unit of conduit, (2) that mature sieve-tube protoplasm is in a state of reduced activity, and (3) that phloem exudation occurs at rates sufficient to explain common phenomena of growth and storage.

Since the perfect experiment for demonstrating which mechanism actually functions in the phloem has not been devised, judgment must rest, for the present, upon a reconciliation of the recognized facts with known plant structure and behavior. With this in view, new observations on the properties of sieve tubes will be presented, and an attempt made to interpret current research on food movement.

Phloem studies

Many observations emphasize the important relation between ontogeny and function of the phloem. Previous studies (**12, 13, 14, 15**) have indicated the changes in form and physiology that take place as the sieve tube matures, passes through its functioning period, and is obliterated. Recent observations confirm these earlier reports.

Young sieve-tube elements are spheroid or cylindrical and well rounded. Having nuclei, slime bodies, and cytoplasm in a high state of activity, they react to stains and plasmolyzing solutions as normal vacuolated living cells. Attending the nuclear disintegration, loss of slime bodies, and alteration of permeability that occurs with maturity, are morphological changes of profound significance (**12, 13, 14, 15**). At maturity the side walls of sieve tubes are slowly pressed in by surrounding cells so that the elements become funnel-shaped at each end, the transverse area in the center becoming progressively smaller. Finally the walls are pressed together, and the lumen disappears completely; the only structures left at obliteration are the sieve plates, often found lodged between the phloem parenchyma cells. ESAU (**26, 27, 29**) has illustrated this process of sieve-tube obliteration in the formation of the bundle cap in sugar beet and celery. Though specific studies have been made on only a few species, this general behavior of sieve tubes is well known and has been observed by plant anatomists since the pioneering studies of the past century. It apparently provides almost indisputable evidence that the sieve tube becomes permeable, loses turgor, and is finally collapsed by the pressure of surrounding phloem parenchyma cells (**6, 27**).

ESAU (**29**), studying several plants, has noticed that when phloem tissues

are killed by reagents which plasmolyze living cells, young sieve tubes always show shrinkage of the cytoplasm, indicating that they were plasmolyzed in the killing process before the tissue was sectioned. Mature sieve tubes, on the other hand, do not respond to these reagents in this way. As she has pointed out, STRASBURGER made the same observation years ago.

Another unique feature of mature sieve tubes is that the plastids, instead of being associated with the peripheral cytoplasm, are free, floating in the vacuoles (**13, 14**). This fact again indicates an altered vitality of the cytoplasm, since in all normal living cells the plastids remain attached in some way. The separation of the plastids from the cytoplasm occurs at maturity, when the inner bounds of this layer appear to fade and become indefinite (**13**).

These many observations, which involve intact as well as sectioned material and which in part date back to the earliest studies on phloem anatomy, indicate with increasing conviction that sieve tubes undergo fundamental changes in their osmotic relations as they reach maturity and are finally obliterated. According to the most logical interpretation, they lose their property of semipermeability and are slowly crushed by surrounding parenchyma cells that survive them by an appreciable period of time. Persistent differentiation and ontogeny of tissue are characteristic of the phloem, and so long as life remains in a plant a certain number of maturing sieve tubes are available to serve their function in conduction.

PHILLIS and MASON (**54**) object that the highly permeable condition of the mature sieve tube may be an artifact resulting from sectioning. More recently, MASON and PHILLIS (**48**) state: "In order to insure plasmolysis of the sieve tube it is necessary to carry out the operation on the bark *before it is cut* and removed from the plant." They do not explain how they arrived at this conclusion; and, since the necessary microscopic observation would require some dissection, one cannot easily understand how the work could be done critically. To be certain of his observations one should carry out the plasmolysis under the microscope, where the condition of the sieve tube before and after treatment could be observed. Only in this way can one determine the ontogenetic state of the tube, its freedom from injury, and its reaction to the treatment.

CURTIS objected, several years ago, stating that the permeable condition of the mature sieve tube might be an artifact, but he was unable to substantiate his hypothesis by experiment. Although this point cannot be disregarded, one should remember that the living protoplasm of young sieve tubes and of phloem parenchyma cells is not seriously altered by the injury attending sectioning. Protoplasmic streaming, vital stain accumulation, and plasmolysis occur in these cells despite the sectioning process. These cells are connected by protoplasmic connections as are mature sieve tubes.

There is at present no reason for assigning a special sensitivity to mature sieve tubes. Certainly the evidence indicates lowered rather than increased activity and responsiveness of these cells to injury or manipulation.

Slime plugs in sieve tubes have been studied since the earliest investigations of phloem and have been cited as evidence both for and against a mass flow. Recent observations have explained many of the difficulties in interpreting the relation of slime plugs to translocation. According to a late paper (**15**), although such structures may be found lodged against the distal sides of sieve plates for several centimeters from the cut end of a cucurbit stem, removal of a thin slice 1 mm. or less in thickness would cause phloem exudation to be resumed. Slime plugs so located are particulate in structure and consist of the decomposition products of the slime bodies and nuclei. An earlier publication (**12**) cites instances where slime plugs were formed in different regions in sieve tubes by treatment with heat, alcohol, and other coagulating agents. Such plugs are usually amorphous. They consist of the normal sieve-tube sap, which can be coagulated either inside or outside the sieve tube by the same agents.

The particulate slime plugs therefore indicate a mass flow of solution through the sieve tubes, being composed of material filtered out at the plates. Studies on these structures and on the nearby sieve plates give no indication of perforations within the protoplasmic strands traversing the plates. The amorphous plugs are composed of coagulated sieve-tube sap; and according to studies recently reported (**15**), this sap may continue to flow from cut stems for periods of 24 hours, coming from great distances along the stem. *The sieve tubes must be permeable to this sap, or it could not continue to flow from cut stems for such periods.*

Mason and Phillis (**48**) question the source of the phloem exudate from cucurbits. Repeated studies under the dissecting binocular prove that it comes from the phloem, and with high magnification and well-developed stems it appears to flow from cut sieve tubes. If it does not come from sieve tubes, then the phloem parenchyma must also have some special organization allowing a ready flow of "sugars, nitrogen, phosphorus, potassium, magnesium, and chlorine" and water across them (**48**). The plasmolytic properties of these cells do not indicate such an organization.

Much confusion has resulted because this coagulable material does not occur in all sieve-tube sap. Products of slime-body and nucleus disintegration, on the other hand, occur in all primary sieve tubes at certain stages in their ontogeny and may in their final stages of disintegration approach (for instance, in the potato plant) the amorphous form of the coagulable sieve-tube sap of cucurbits and similar plants. Unless, however, this amorphous material can be demonstrated in mature elements and coagulated outside the sieve tubes, it must be considered only transitory, and not an essential part of the plant-nutrient supply.

The presence or absence or location of slime plugs depends, therefore, not alone on translocation but also upon the stage of sieve-tube development, the species of plant, and the agents used for demonstration. Without a full knowledge of these matters one cannot correctly interpret the relation of slime plugs to translocation.

Dissection studies on cucurbit phloem reveal some important properties of sieve-tube cytoplasm. Whereas the cytoplasm of phloem parenchyma in this tissue is fragile and easily torn and fractured, the peripheral layer of protoplasm in the sieve tubes is tough, ductile, and somewhat elastic. As seen in dilute water-blue solution, the protoplasmic sheath of mature sieve tubes can be drawn out to many times the length of the element in which it originally occurred. When released it rapidly shortens, but not to its initial length. When stretched to the breaking point it often tears loose from the sieve plate and comes away as a long threadlike strand.

By removing a tangential slice from an intact squash stem, laying bare the phloem, one may observe by reflected light the coagulation of the sieve-tube sap in the tubes. This occurs most rapidly in the cut elements but takes place slowly in deeper layers. Obviously, such a coagulation within the interstices of the phloem walls would increase resistance immensely and stop flow.

If uncut sieve-tube elements are quickly flattened with the dull edge of a scalpel, the sieve plates are deformed, the cupped shape being reversed if the pressure is so increased on the convex side. This fact indicates resistance to extremely rapid flow. The sieve plate of the succeeding element shows little response, however, to this rapid change in pressure; and plates two or more elements away show no reaction. Particulate material in the separate elements does not pass the plates but is filtered out. These observations are further evidence for the relative permeability of mature sieve tubes to dissolved substances.

In connection with the measurements of phloem reported in the following section, a few studies were made on cotton sieve tubes. In common with other species examined, the young sieve-tube elements accumulate neutral red and plasmolyze with strong sucrose solution; but mature elements cannot be so plasmolyzed. Slime bodies were observed in young elements; but these disappeared, along with the nuclei, at maturity. Plastids in the maturing elements may contain several starchlike grains that stain wine red with iodine. In older elements the small starch-grainlike bodies occur free within the vacuole, agitated by Brownian movement. Each sieve-tube element of the Acala cotton plants studied contained a small but conspicuous body, having an amorphous core and a thick coating with oddly pitted sculpturing. The phloem parenchyma cells of the stems and leaves had plastids containing chlorophyll in low concentration.

The evidence presented above, though largely circumstantial, indicates that the sieve-tube protoplasm undergoes some marked changes during maturity and that the function of the phloem is most probably involved.

The next studies are concerned with the quantitative relations of the "activated diffusion" hypothesis. Although the Trinidad workers[3] have produced a large volume of data concerning the gross responses of cotton plants to ringing and other similar manipulations, it does not follow that these data substantiate the "activated diffusion" theory. In fact, their own calculations on rates (**41, 42**), so widely quoted, may not be in accord with these data.

Quantitative aspects of the "activated diffusion" hypothesis

By rather devious reasoning the Trinidad workers have concluded that solutes move through a "relatively stationary medium" (**44**) in the sieve tubes by a process termed "activated diffusion" (**45**). In several places they compare this with polar concentration or absorption across protoplasmic membranes. They point out that both processes depend upon the metabolic activity of cells and utilize energy in performing work. They fail, however, to consider that one is taking place over centimeters and even meters, the other only across microns. Although energy is expended in transferring ions or molecules from the exterior to the vacuole of a cell, or from vacuole to vacuole against a concentration gradient, diffusion is moving these materials through walls up to the cytoplasmic membrane and in most cases probably acts as a limiting factor in controlling rates.

The problem of longitudinal movement through sieve tubes is entirely different. MASON and MASKELL give rates as high as 0.6423 gm. per square centimeter per hour for movement along what they term "sieve tubes" (**42**, p. 629). Normal rates vary from 0.14024 (**41**, p. 247) to 0.2356 (**42**, p. 629). Now, these rates are based on "the whole area of the sieve-tube groups in the phloem, and include companion cells as well as the walls of the tubes" (**41**, p. 248). Evidently the total transverse area of the inner phloem has been used for computing these values.

Measurements recently made on the phloem in stems of Acala cotton indicate the relation among areas of phloem walls, companion and parenchyma cell lumina, sieve-tube lumina, and sieve-tube cytoplasm. A stem having a transverse area of 2.74 cm.^{2} was used, measurements being made on the bark at a point about 4 inches above the soil level.

The bark of cotton consists of groups of phloem, surrounded on all but the cambial side by parenchyma cells of the rays and cortex. The phloem groups consist of intermittent bands of sieve-tube-containing groups and fibers. Fresh sections of these tissues mounted in water were projected

[3] MASON and his associates.

and traced on paper. Then the sieve-tube groups were drawn under the camera lucida, and the drawings and tracings were measured by cutting out and weighing the various areas. Table I shows measurements on tissues and regions of the stem and peduncle.

An oil-immersion lens with critical illumination, used with an ocular micrometer, showed the cytoplasmic layer of the sieve tubes to be approximately 0.2 μ thick. By means of this value and the average sieve-tube areas found (table I), areas for the sieve-tube cytoplasm were calculated. These values, expressed as areas and also as percentages of the actual sieve-tube areas and "sieve-tube-group" areas, appear in table II. A weighted average of the latter values turns out to be 0.98 per cent. Apparently, therefore, the sieve-tube cytoplasm occupies about 1 per cent. of the total area of the sieve-tube groups used by MASON and MASKELL as a basis for calculating rates.

TABLE I

MEASUREMENTS SHOWING RELATIVE AREAS OF VARIOUS TISSUES AND REGIONS IN COTTON BARK. THE TERM "SIEVE-TUBE GROUP" IS USED IN THE SENSE OF MASON AND MASKELL AND INCLUDES ALL PHLOEM EXCEPT THE FIBERS

TISSUE	AREA	PERCENTAGE OF TOTAL
	CM.^{2}	STEM
Bark	0.766	27.8
		Percentage of bark
Phloem	0.314	40.8
Cortex	0.452	59.2
		Percentage of phloem
Sieve-tube groups	0.180	57.3
Fibers	0.134	42.7
SET 1: 4 PHLOEM GROUPS WITH 66 SIEVE TUBES, FROM THE ABOVE STEM		
	cm.$^2 \times 10^{-4}$	Sieve-tube groups
Sieve-tube lumina	0.98	13.7
Companion and parenchyma cell lumina	3.97	55.1
Phloem walls	2.24	31.2
SET 2: 7 PHLOEM GROUPS WITH 62 SIEVE TUBES, FROM THE ABOVE STEM		
Sieve-tube lumina	0.76	9.9
Companion and parenchyma cell lumina	4.82	62.5
Phloem walls	2.12	27.5
SET 3: 3 PHLOEM GROUPS WITH 172 SIEVE TUBES, FROM PEDUNCLE		
Sieve-tube lumina	0.71	10.9
Companion and parenchyma cell lumina	3.31	51.5
Phloem walls	2.42	37.5

It seems inconceivable that the accelerating mechanism postulated by these workers can act at any great distance from the sieve-tube cytoplasm. Sugar movement along the cytoplasm must take place, therefore, at rates in the order of 100 times those quoted if based on the total area occupied by protoplasm in the sieve tubes. If 50 per cent. of the area is occupied by the permanent cytoplasmic structures and by water of hydration (80 per cent. would probably be a more rational value), then obviously sugars

TABLE II

AREAS OF SIEVE TUBES AND SIEVE-TUBE CYTOPLASM IN COTTON BARK

SET NUMBER	AVERAGE SIEVE-TUBE AREA	AVERAGE AREA SIEVE-TUBE CYTOPLASM	CYTOPLASM AS PERCENTAGE OF SIEVE-TUBE LUMEN	CYTOPLASM AS PERCENTAGE OF SIEVE-TUBE GROUP
	μ^2	μ^2	%	%
1	148.4	8.4	5.7	0.78
2	122.6	7.8	6.4	0.63
3	41.3	4.5	10.9	1.19
Weighted average for 300 tubes				0.98

must move at rates of 200 (to 500) times those originally proposed by these workers (**41, 42**). The mechanism of VAN DEN HONERT (**63**) and the suggestions of CLEMENTS (**8**) and LEONARD (**33**) are open to similar quantitative criticism.

The work so far described indicates that the protoplasm of mature functioning sieve tubes has unique characteristics. Apparently it exists in a low activity state, being relatively permeable, without streaming motion, and of a tough, ductile nature.

The quantitative interpretation just presented shows the extreme linear rates of unilateral movement necessary to provide for known translocation, and indicates the inadequacy of a purely diffusional type of transport.

In the final analysis, the protoplasmic theories all depend upon a supposed independence of movement of various solutes in the phloem.

Since organic materials are commonly synthesized in localized regions and subsequently moved, obviously gradients in the various forms must occur. But the mere existence of a concentration difference between two plant tissues or organs does not prove that translocation is occurring or normally does occur between them (**33, 36, 39**). In the translation of concentration gradients to actual movement over any distance, MÜNCH's mechanism (**50, 51**) is particularly significant, for only where a physical mechanism exists can gradients be related to rapid movement.

CURTIS (**22**) and MASON and PHILLIS (**46**) question MÜNCH's mechanism because it will not explain simultaneous movement of solutes in opposite

directions through the phloem, a process which they claim to have demonstrated. The evidence presented for this simultaneous movement is not, however, particularly convincing.

Evidence for independent movement

CURTIS interpreted his early experiments (**17, 18, 19, 20**) as indicating a simultaneous upward movement of nitrogen and downward movement of carbohydrates through the phloem. His selection of the mechanism of DE VRIES—namely, diffusion through the phloem accelerated by protoplasmic streaming—to explain movement of all solutes seems to have resulted from this interpretation. Apparently he did not consider, at the time, that a mass flow of organic solutes, including organic nitrogen compounds, might have been taking place upward through the phloem (**22**, p. 56). His own data, showing large differences early in the season, when phloem movement would have been predominantly upward, and smaller differences later, point to this very possibility. In none of these experiments did he prove a downward movement of carbohydrates.

CLEMENTS and ENGARD (**10**) have shown that nitrogen absorbed from the soil can move into the top of the plant through the xylem (**7**), and CURTIS failed to demonstrate complete stoppage of nitrogen movement by ringing. His experiments, therefore, do not prove independent movement of nitrogen and carbohydrates in the phloem.

Recently the Trinidad workers have attempted to demonstrate independent movement of solutes through the phloem by special experiments (**44, 46**). Using mass analysis of leaves of ringed and unringed cotton plants and testing for nitrogen and carbohydrates in shaded and unshaded leaves, they tried to show a simultaneous movement in opposite directions through petioles and stems. Their first experiment failed, both solutes moving together. They did prove, however, that the movement of these materials could be reversed. Their second experiment showed that xylem movement will adequately explain the upward transport of mineral nitrogen. Though there is some question concerning their determination of carbohydrates by difference between two variables, in the study on movement of stored nitrogen (third experiment) a more serious weakness is the time interval between samplings. Their own earlier work indicates the effect that ringing may have upon xylem transport after an initial period of about 12 hours. Their evidence for independent movement in the stem in this experiment is very inconclusive. The results, furthermore, are subject to a completely different interpretation, a weakness that they fully recognize (**44**, p. 50).

In their final experiment MASON and PHILLIS (**46**) attempted by shading and ringing to demonstrate a simultaneous movement of carbohydrates and

storage nitrogen through the phloem of cotton. Their results show a net gain in carbohydrates and a loss of nitrogen in shaded as compared with illuminated portions of single plants and show also that these changes were prevented by a ring between the respective regions. To get significantly different changes, however, they made their sampling periods all 48 hours or more in length; and apparently they failed in their interpretations to recognize the possibility of a diurnal reversal of pressure flow through the phloem.

In their earliest experiments with cotton, MASON and MASKELL demonstrated a diurnal variation in translocation rate. Subsequently they have shown that movement is polar with respect to concentration gradients within the phloem and that by reversing the gradients one can reverse solute movement. In the study under consideration (**46**) photosynthesis would predominate during the day, causing a downward movement of carbohydrate from the illuminated leaves to the darkened ones. These, being low in carbohydrate, would accumulate it as long as movement continued. When photosynthesis stopped and the gradient flattened, the use of carbohydrates in respiration would allow the pressure to drop to a place where the gradient of hydrolyzed nitrogen compounds in the shaded leaves would cause a slow reversal of flow such as has been demonstrated for autumn leaves. Nitrogen would be accumulated by the high-carbohydrate leaves above. The experiment as performed could not have been more perfectly designed to demonstrate this effect, and such an interpretation seems as logical as that of the authors.[4]

Repeated attempts by able workers have not yet adequately demonstrated independent movement of solutes in the phloem. Some experiments performed (**44**) have, in fact, shown the opposite, namely, that when carbohydrates and nitrogen move simultaneously in the phloem they go in the same direction and that when they go in opposite directions they do so in separate tissues (**44**). Virus studies (**2, 3, 4**) substantiate this observation.

In this connection, SHUMACHER (**60**) found in studying *Cuscuta* that fluorescein introduced into the phloem of the host was taken up by the parasite with sugars and other foods and that these materials moved together in the phloem of the parasite. MASON and PHILLIS found (**47**) that

[4] GUSTAFSON and DARKEN failed to prove independent movement; upward movement of phosphorus was slow, simultaneous downward movement of carbohydrate was not measured. GUSTAFSON, FELIX G. and DARKEN, MARJORIE. Further evidence for the upward movement of minerals through the phloem of stems. Amer. Jour. Bot. 24: 615–621. 1937.

PALMQUIST failed to prove fluorescein moved at rates exceeding protoplasmic streaming; carbohydrate movement was likewise not accurately measured. PALMQUIST, EDWARD M. The simultaneous movement of carbohydrates and fluorescein in opposite directions in the phloem. Amer. Jour. Bot. 25: 97–105. 1938.

not only sugars but also phosphorus, potassium, magnesium, and chlorine respond to influences affecting movement in the phloem. Calcium was immobile after its primary movement to the leaves through the xylem, most likely because it combined with oxalate to form the crystals common in the phloem of cotton.

Until independent movement can be shown, all the critical experiments on translocation can be more adequately explained on the basis of unidirectional flow through the phloem or xylem. To explain pressure flow through both xylem and phloem, furthermore, we have at hand mechanisms that are fairly compatible with present physical concepts.

Discussion

The diversity of opinion on the mechanism of food movement in plants results from several causes. In going through the immense volume of data available on the subject, one is impressed by the differences in interpretation that occur. Although the grosser responses of plants have been well demonstrated, there is an obvious gap between the experimental work and the final conclusions. It seems timely to consider briefly some of the reasons for this.

Experimental materials

In translocation studies a wide variety of plants have been used; and, as a rule, generalized interpretations have not attached sufficient weight to inherent differences occurring among them.

Except for certain horticultural varieties, trees in general grow slowly in relation to their mass. Spring vegetation may be largely produced from stored reserves; summer growth is nourished from current supplies; and products of assimilation may be stored largely during the latter part of the season.

With the herb, vegetation starts from reserves in the seed but soon becomes self-supporting. In many species materials may be stored in specialized organs; in others the bulk of assimilated products goes to produce the plant body. Since these different behaviors make different demands on the vascular organs, translocation must proceed at vastly different rates. Besides, plants of like form may vary widely in the types and amounts of materials moved and stored, and in the rate and reversibility of movement.

Although materials must move great distances in trees, related processes go on slowly; rapid translocation probably does not occur. In vegetative herbs such as tomato and tobacco (**14**), whose stems and even fruits may contain chlorophyll, products of assimilation are largely used locally; again high rates of movement are not required. For critical studies involving

changes, rates, and gradients, specialized plants having rapid translocation should be used. For such studies the potato (**13**), the yam (**40**), the sugar beet (**5**), and certain cucurbits (**12, 15**) are favorable. Although not all studies need be limited to these plants, where diurnal variation and incipient changes require frequent sampling to obtain significant differences such plants should be included in any comprehensive work. Processes like xylem and phloem exudation from cucurbits and translocation into potato tubers, beet roots, and cucurbit fruits take place at high rates and are subject to accurate measurement over short time-intervals. Data on these processes should be critical. To state that they are not normal is to beg the issue. Rates of movement in such plants are necessarily high. If interpreted with due regard for the experimental technique involved, they should give a clear picture of the processes at their upper limits.

Though plants may vary widely with respect to rates of movement through the phloem (**4, 11, 12, 13, 15, 41, 42**) there is, however, nothing in the structure of different forms to indicate that different mechanisms of movement are involved, as suggested by CURTIS (**22,** note at foot of p. 79). Plant anatomy affords strong evidence that the underlying mechanics of translocation in both the xylem and phloem are the same in all species. Variations in behavior apparently relate more directly to differences in rates of synthesis, translocation, and utilization than to differences in the mechanics of movement.

PRIMARY AND SECONDARY MOVEMENTS

Another source of difficulty is the fact that movement of various materials is taking place simultaneously in different tissue systems within the plant body and is activated by different mechanisms. Unless the distinct nature of these is recognized, interpretations may be confused.

Although secondary processes of translocation are often intricate and difficult to study and visualize, certain primary functions are obvious. Mineral nutrients, for instance, are absorbed by the roots and are ultimately distributed throughout the plant. Organic compounds synthesized in green tissues are subject to the same general distribution. In considering translocation, especially with relation to gradients, one should not overlook these facts.

Besides primary movements there is evidence for others. To a certain extent in all plants and very largely in woody plants, absorbed nitrogen combines with organic material soon after entrance to form amide, amino, and other reduced-nitrogen compounds; and nitrogen may move largely in these forms, organic radicles being retranslocated to the foliar regions. As CURTIS and others (**22, 33**) have pointed out, there seems little justification for MASKELL and MASON'S assumption that "the bulk of the mineral

nitrogen absorbed by the roots is carried in the transpiration current to the leaves, where it is elaborated . . . and exported through the sieve tubes back to the roots" (**37**). On the other hand, as MASON and MASKELL (**43**) have shown, nitrogen, potassium, and phosphorus may move out of leaves and be distributed throughout the plant via the phloem. Hence secondary movements of both organic and inorganic nutrients may be important in the general process of translocation, the relative rôle of each depending upon rates of absorption, synthesis, and utilization.

A problem often overlooked is the lateral distribution of nutrients from vascular tissues. Since the ultimate distribution of all materials is generally throughout the plant body (**30**), all nutrients translocated but not used by the vascular tissues themselves are distributed laterally from these tissues; and since the nutrients are largely used by meristems to produce new tissues, processes of growth and translocation are necessarily closely related. Nutrition of meristems is an important function of vascular tissues; and the supply of nutrients to growing cells is apparently not a mere "leakage," but an active movement carried on by the intervening protoplasm. A dynamic equilibrium should exist between the cambium and solutions moving in the adjacent xylem and phloem tissues, growth being conditioned largely by the balance and supply of foods.

Although growth and translocation are closely interrelated, the latter providing food for cell expansion and wall formation, and although growth fixes foods in non-mobile and less active forms so that the growing tissues act as "sinks" for all foods, there is seemingly no evidence that foods are moved for any great distance by the growth process or move through growing cells (**56**). Differentiation and growth are continuous functions of active phloem tissues; within the phloem there are maintained constantly a number of mature sieve tubes. That growing sieve tubes are active in translocation does not, however, necessarily follow. According to our present knowledge, conduction should be assigned to the mature elements, growth being an effect rather than a cause of the movement and supply of foods (**56**).

PROTOPLASMIC CONDUCTION

Turning now to the actual paths of movement, there are apparently two mechanisms involved in the final distribution of foods. First, there is a slow movement along the cytoplasm from the chlorenchyma to the phloem, and again from the phloem out to the final destination in meristems, storage, or other utilizing cells (**13**). This is pictured as a diffusional movement accelerated by protoplasmic streaming and wherever the longitudinal rate of transport does not exceed that of protoplasmic streaming it seems that this mechanism suffices. The rapid longitudinal transport of foods at rates of 20 cm. per hour or more is a different sort of process and requires a

different mechanism. Such movement must occur principally in the sieve-tube lumina.

SCHUMACHER considers that his experiments with fluorescein (**59, 61**) prove a rapid movement of this dye through sieve-tube cytoplasm. There are two other possible explanations of his observations. In 1932 the writer suggested (**12**) that eosin in SCHUMACHER's early experiments (**58**) was being absorbed into and carried along the xylem, reentering the phloem at points removed from that of its original application (**12,** p. 195). RHODES presents a similar interpretation of fluorescein movement in a recent paper (**57**; see also **47**), but SCHUMACHER in repeated experiments, including some in which the xylem from *Pelargonium* petioles was removed, seems to demonstrate that fluorescein may move in the isolated phloem (**61**).

Granting that fluorescein moves rapidly in the phloem (**59, 61**), without some definite information on the partition of this dye between the cytoplasm and vacuole it is questionable to attribute the actual transport to the cytoplasm. There is still some doubt as to the localization of fluorescein in the sieve tubes in SCHUMACHER's experiments (**59**). One might assume a rapid movement of the dye in low concentration through the vacuoles with adsorption by the cytoplasm to explain his observations. Other dyes, such as aniline blue, are accumulated in the cytoplasm of mature sieve tubes from a low external concentration. Regardless of the path of movement of fluorescein, however, sugars, which move in vastly larger amounts through the phloem, would have to overcome extreme resistance to travel at the required rates through cytoplasm.

Continued experiments on chilling and coating petioles (**21, 24, 47**) have failed to prove that sieve-tube protoplasm plays an essential rôle in longitudinal transport. Reduction in rates may be explained on the basis of viscosity change and altered permeability of phloem tissues. This type of experiment is of doubtful value in the final solution of the problem.

According to MASON and PHILLIS (**47,** p. 497), "The mechanism activating diffusion (in the sieve tube) consists of some special organization in the cytoplasm, maintained by metabolic energy, whereby the resistance to solute movement is so reduced that materials diffuse in the sieve tube at rates comparable with those in a gas." For reasons unknown, these writers make an illogical choice in selecting the solute as the object of this special mechanism. Experiments on water and solute absorption, plasmolysis, and water movement in tissues indicate that the solvent, water, moves through protoplasm more readily than do solutes. If water is included among the substances that move by "activated diffusion," then there is little difference between the "activated diffusion" and the pressure-flow theories, for movement in the latter instance is a special case of diffusion of solvent and solute through tissues along a concentration gradient of solution. Such flow must

occur through the walls of root hairs, possibly through the vacuoles of endodermal cells, and through the walls of xylem vessels in roots that are absorbing water and solutes simultaneously. The apparent independence of solute movement under these conditions might result from differential resistance to various solutes by the membranes traversed, such resistances being related to molecular sizes, hydration, and the like. Whereas the pressure-flow theory pictures acceleration in the sieve tube as resulting from a hydrostatic pressure, developed osmotically and exerted impartially upon solute and solvent, the "activated diffusion" theory requires a selective application of energy to the solute molecules by the protoplasm. The former is based upon almost innumerable observations of phloem exudation where solute and solvent flow from cut phloem in volumes entirely too large to have come from the cut elements alone (**15**). The latter is entirely hypothetical and rests largely upon the questionable evidence for independent movement that was considered in a previous section.

SCHUMACHER, discussing the movement of fluorescein in cucurbit (**59**), states that he plasmolyzed sieve tubes with a sucrose solution. Evidently he was observing young sieve tubes. His studies on exudate, however, must have involved mature elements. Apparently he overlooked the importance of sieve-tube ontogeny in this problem and consequently misinterpreted many of his observations.

MOVEMENT ALONG CELL WALLS

MASON, MASKELL, and PHILLIS have objected to my postulation of flow along phloem walls. Although I have altered my views on this subject (**15**), it still seems probable that movement through wall material takes place (1) from the furthest differentiated sieve tubes to the growing points of root and shoot, (2) across sieve plates from element to element, and (3) laterally when gradients are altered by such practices as partial ringing (**42**) and sectional pruning.

Considering for the moment that when a healthy plant cell is plasmolyzed with sucrose, the molecules may diffuse through the wall in a matter of minutes, whereas they may take hours or even days to traverse the cytoplasm, flow along walls does not seem irrational compared with movement at even higher rates through cytoplasm. If the cytoplasm of mature sieve tubes is permeable (**13**), this objection is less pertinent. It seems highly unlikely, however, that protoplasm devoid of streaming movements and completely permeable to solutes could be the seat of such high metabolic activity as would be required to provide the necessary acceleration. Rather, protoplasm of the functioning sieve tubes seems to be definitely degenerating and passing into a condition of prolonged senility, followed by death (**12, 13**).

Inadequacy of mass analysis

Many interpretations by the Trinidad workers and others indicate the inadequacy of mass-tissue analysis for studies on translocation. Curtis has pointed out this weakness (**22**). Granted that photosynthesis occurs in the plastids and that movement of carbohydrates to the sieve tubes takes place by diffusion, accomplished via the protoplasm and accelerated by streaming (**13, 22, 50, 51**), there is apparently no reason to postulate a polar accumulation by transition or companion cells, and secretion into the sieve tubes (**53**). One might more logically assume that carbohydrates are high in concentration in the cytoplasm of mesophyll cells and move along a positive gradient into the sieve tubes. Mass analysis gives predominantly a picture of vacuolar concentrations and indicates simply the amount of solutes maintained in the vacuoles of mesophyll and cortical parenchyma cells to preserve their form and to insure them against desiccation. Vacuolar concentrations in the sieve tubes are in equilibrium with those of the cytoplasm because of the peculiar permeability conditions; and there alone may the true status of nutrients within the actual paths of movement be measured. Until methods are developed for determining sugars in the cytoplasm and in sieve tubes, it seems futile to interpret mass analyses in terms of translocation. Loomis's assumption of polar movement of carbohydrates in maize (**35**) is based upon such faulty interpretation. If one does not assume that food movement determines fertilization, Loomis's defoliation and defruiting experiments all fit the mass-flow theory. Gradients as determined by mass analysis reflect vacuolar concentrations in the various tissues analyzed. Although they indicate relations of storage and water loss, they form no adequate basis for the assumption of polar movement.

According to recent experiments on translocation in the sugar beet (**5**), phloem parenchyma may play an important rôle in maintaining necessary gradients by temporary storage of sugars; and the results reported by Phillis and Mason (**53**) are probably more directly related to this effect. This suggestion, however, should be checked by analyses showing vacuolar concentrations in individual cells.

As this discussion shows, more detailed information on tissues involved in absorption and transport is needed for an understanding of the mechanics of these processes. Mass-tissue analysis gives an excellent picture of the gross responses of plants to ringing (**22, 34, 36, 41, 42**) and similar practices. Workers, with few exceptions (**22**), agree that the primary upward movement and distribution of mineral nutrients absorbed by the roots take place through the xylem and that organic materials synthesized in the leaves pass to utilizing cells through the phloem (**52**). The final agreement upon the mechanics of movement in sieve-tubes, however, must await the development of methods for studying single tissues and possibly single cells.

Conclusions

The translocation problem is obviously complex. At least two tissue systems are utilized in actual conduction; all tissues of the plant and most functions are involved directly or indirectly. Only by viewing the problem in this broad aspect may one hope to integrate its various phases into an organized mechanism and to harmonize it with known plant structure.

Root absorption by higher plants, involving as it does accumulation by single cells, secretion into nonliving ducts, and movement both to and away from the functioning organs (**16**), constitutes the primary process in the translocation of mineral nutrients and water (31). Absorption and translocation by roots seem, in fact, inseparably related and should be studied as phases of a single phenomenon.

In dealing with the problems of absorption and translocation we cannot neglect structure. There is, for example, no apparent reason for assuming a general upward movement of nitrogen in the phloem, simultaneous with the downward passage of carbohydrates. Root anatomy apparently indicates much greater difficulty in absorption by the phloem than in release into the xylem (**11, 16**). No mechanism in the phloem explains the extremely rapid rise of minerals observed in the plant. Many reports on the occurrence of both inorganic and organic nitrogen compounds in xylem sap indicate their presence in the water-conducting tracts (**1, 55, 62**). This general knowledge, with the results of MASKELL and MASON (**37, 38**), HOAGLAND and BROYER (**32**), CLEMENTS and ENGARD (**10**), and others, points to conduction of mineral nutrients in the xylem.

Viewing mineral movement in relation to anatomy and considering the rates and distances involved, one may conclude that the roots are the primary source of minerals in the plant; that the minerals may diffuse laterally from the xylem during their upward movement; and that any residual minerals not held by the leaves may be retransported via the phloem with carbohydrates. The leaves would thus constitute not only a portion of the sink for minerals in primary movement but also the source of the secondary supply. This flow of carbohydrates and residual mineral nutrients in the phloem is apparently the most important supply to the primary-shoot meristems.

In certain herbaceous plants plentifully supplied with nitrogenous fertilizer there may be a large secondary movement of nitrogen out of the leaves. In many plants, however, especially in woody ones, the primary movement would predominate; and the secondary movement might cease entirely, at least during the latter part of the growing season. Evidently a secondary release from most leaves occurs before death or under heavy shade.

Data on problems of translocation are rapidly accumulating. New ob-

servations have been made on phloem exudation (**2, 3, 4, 15**).[5] Although this evidence is not complete, each added contribution helps. Plants vary widely in their rates and volumes of phloem exudation. In view of their forms and functions, this variation seems rational. Highly vegetative herbs, as pointed out, need not move organic foods over great distances. Trees, because of relatively slow growth over long periods, also have low requirements except in fruit formation. Only plants that rapidly store large quantities of carbohydrate require high rates—for example; cucurbits and sugar beets, which exhibit rapid phloem exudation.

The pressure-flow mechanism should meet certain functional and structural requirements. Turgor gradients pictured by MÜNCH have been proved nonexistent (**22, 23, 53**). The sugar-beet plant is an excellent example, having 5 to 10 per cent. sugar in leaf cells, about 10 per cent. in phloem exudate, and as high as 25 per cent. in root storage cells. Both the diffusion and pressure-flow theories require, apparently, accumulation within certain receiving cells to concentrations higher than those of the conducting tissues to provide the necessary concentration gradients.

Whereas WEEVERS and WESTENBERG (**64**) were unable to collect water of exudation from the cambium by the method of MÜNCH, MOLOTKOVSKII (**49**) was apparently successful. He agrees with MÜNCH that this water comes from the cambium and suggests that the sugar appearing during the first few days comes from injured phloem. Organic materials appearing later in the experiments come from contaminating fungi. The occurrence of this type of exudation probably depends upon a relatively abundant supply of water to the tree. A demonstrable flow is not essential to the pressure-flow theory, for the water accompanying foods in the phloem may be largely used in growth or even lost by evaporation through the bark. True phloem exudation is the most important prerequisite; and where it fails entirely, either translocation is not taking place or some other mechanism is involved. Recently reported xylem-exudation studies show another weakness of the MÜNCH hypothesis. MÜNCH'S explanation of root pressure as resulting from unilateral secretion of water from the phloem will not explain the large volumes nor the ion concentrations of exudate from excised root systems. His view of sieve-tube structure has, furthermore, proved untenable, in the light of more recent studies. There seems to be no reliable evidence for intervacuolar passages within the protoplasmic strands of the sieve plate.

As critics of the pressure-flow theory have pointed out, phloem exudation varies among species and may not be demonstrable in some. Demonstration of this phenomenon, however, depends upon several factors. If any appreciable volume is to come from a cut stem, rapid translocation must

[5] See also footnotes 2 and 6.

be in progress, and exudation must exceed evaporation. Capillary absorption by intercellular spaces of the cortex and pith must be prevented. Flow, furthermore, must be rapid compared with stoppage. In order to survive, plants must have developed a mechanism for preventing excess leakage. A recent paper reports observations on this phenomenon (**15**). The relative rates of flow and stoppage determine the volume of exudate that comes from a single cut. The best known way to maintain flow is to repeat the cutting (**11, 12, 13, 15**). By proper technique, phloem exudation may eventually be demonstrated in all higher plants.[6]

The physiological-anatomical relations of phloem transport form a fertile field for study. Much should be known concerning the limiting layer that retains, within the phloem strands, materials in transport. Dissection experiments (**11**) indicate such a layer. Though the relation of virus movement to translocation of foods is being reserved for a later paper, the recent work on curly top should be noted (**2, 3, 4, 26**). Evidence for movement of virus in the phloem with foods and for exudation of the virus-bearing sap from necrotic phloem through a normally impervious layer seems convincing.

CURTIS has pointed out the difficulties that result from cutting a system composed of nonrigid cells acting under a positive pressure (**22**). A method for artificially injecting such a system without injury would be highly desirable. As BENNETT'S work shows, the inoculation of curly-top virus by the sugar-beet leafhopper provides such a method: though injury results to the phloem, translocation may be rapid enough to carry the virus considerable distances before necrosis becomes serious. On the other hand, the study of internal symptoms has itself proved valuable (**28**), providing additional evidence for the permeability of mature sieve tubes.

The foregoing discussion should show that the divergence of opinion that has characterized translocation study in the past is narrowing and that lack of agreement results not so much from conflicting bodies of data as from differences in interpretation. Workers supporting both the protoplasmic theories and the pressure-flow mechanism assume a specialization of the sieve-tube protoplasm and call upon the activity of living cells. The basic assumption of the former group seems to be that there exists in the phloem rapid transmission of molecules independent of the solvent and related to a high activity state of the sieve-tube protoplasm.

According to the pressure-flow theory, conduction is permitted by a high permeability of the sieve-tube cytoplasm resulting from a decline in its activity state. Cytoplasm in this condition apparently allows a ready passage of food materials in solution.

[6] See recent experiments of MOOSE. MOOSE, CARLETON A. Chemical and spectroscopic analysis of phloem exudate and parenchyma sap from several species of plants. Plant Physiol. 13: 365–280. 1938.

Obviously more research will be required to settle the controversial phases of this problem. It would seem imperative that this research include detailed cytological studies on phloem tissues and that the relation of ontogeny to function be kept in mind.

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ICE FORMATION AND THE DEATH OF PLANT CELLS BY FREEZING

IRENE H. STUCKEY AND OTIS F. CURTIS

(WITH ONE FIGURE)

Introduction

The resistance of plant tissues to low temperatures and the causes thereof have been the subject of extensive research by many investigators. Since there have been comprehensive reviews of both phases of the subject written by CHANDLER (**4, 5**), ROSA (**22**), ÅKERMAN (**1**), MAXIMOV (**15**), LEVITT and SCARTH (**13**), and SCARTH and LEVITT (**23**), this paper refers only to those investigations which are specifically related to the immediate problem.

In all but very few of the previous studies of ice formation in living tissue, the material was frozen by various means, and then after thawing, was examined either grossly or microscopically. What had occurred during freezing was postulated from the appearance of the thawed tissue. Of the few investigators who observed freezing directly, apparently MOLISCH (**17**) was the first to see ice formation as it occurs in living cells. His microscope was put into a specially constructed ice chest with openings to give access to the controls. The apparatus was inconvenient to use, and the temperature could not be regulated, but MOLISCH was able to use it to watch freezing in *Amoebae* and in several plant tissues.

WIEGAND (**26**) investigated the condition of buds during subzero weather. He used a microscope and sectioning apparatus out-of-doors and noted where ice was formed in the buds. Ice was found as a single continuous layer always in the mesophyll of the leaf or scale, never between the scales. The cells on either side were in a state of collapse and often occupied less space than the ice sheet. Ice formation was observed in some species at $-18°$ C., in others not until $-26°$ was reached. This difference seemed to be determined by the size of the cells and the amount of water which they contained. The ice decreased in amount as the temperature rose and finally disappeared at -3.2 to $-2.3°$ C. No injury was noted in buds in which such ice was formed, even after rapid thawing at 21° C.

SCHANDER and SCHAFFNIT (**24**) used a cold chamber mounted on a microscope stage. The chamber was cooled by passing a jet from a tank of compressed CO_2 through ether. By this means the object could be quickly cooled to any temperature down to about $-30°$ C., and the frozen tissue could be observed under the microscope. ÅKERMAN (**1**) used the MOLISCH technique and directly observed the tissues in the frozen condition.

CHAMBERS and HALE (3) made microdissection studies of freezing frog muscle, *Amoebae,* and the epidermal cells of red onion. The operations were performed on prechilled material in a cold room at temperatures ranging from 0° to −5° C.

The methods used by these workers are not entirely suitable for observing the process of freezing. The purpose of this investigation was to find some simpler means for directly observing ice formation in plant tissues, and once this method was established, to apply it to some of the problems concerned with the resistance of plants to low temperatures. We wished to determine more certainly, if possible, where and under what conditions ice occurs, and the relation of water content to resistance.

The observations were made with a Spencer binocular microscope of the type with a square stage and inclined oculars. The square stage was necessary because of the design of the cold stage attached to it. Sufficient magnification was obtained with 10×-oculars; oculars of higher power gave an unsatisfactory image. It was essential to replace the 4-mm. objective of the microscope with one having a greater working distance, consequently the objective used was an achromatic model 4-mm., N.A. 0.65, working distance about 0.9 mm. The top lens of the condenser was removed to raise the point of convergence of the rays of light to the level of the mount in the cold stage. The light source was a microscope lamp with a 250-watt filament bulb, a condenser, and a ground glass.

For the actual freezing process, the cold stage and circulatory system described by MASON and ROCHOW (14) were used. It was found desirable to make a few modifications for best results with biological materials. The bottom cover glass was sealed in place with LePage's cement rather than vaseline. Celotex was used to insulate the stage since sheet cork of the desired thickness could not easily be obtained at the time the stage was made. As stated by the designers of this apparatus, the operation of the system would have been easier if the channels in the stage had been larger, and the circulatory tubes of correspondingly greater diameter. This would allow for greater flow of liquid, and consequently better temperature control. Ether rather than acetone was the liquid used with the solid carbon dioxide in the Dewar flask. A still better method would involve the use of a mechanical water cooler instead of solid carbon dioxide. The plant material was mounted in paraffin oil on an 18-mm. no. 1 cover glass, and over it was placed a fragment of no. 0 cover glass large enough to cover the specimen. It was found easier to mount the material in this way than between two cover glasses. This mount was placed on a tripod of copper wire in the well of the stage. A 25-mm. cover glass which rested on the flange of the stage was placed over it, leaving an air space underneath. The air space insulated the cover glasses, and the objective conducted heat to the well to such an

extent that the material would not freeze when the objective was in focus. To avoid this difficulty, the designers of the stage recommend that the mount be so made that some of the material to be frozen is in contact with the stage; but with the material used, this was not feasible; therefore, solid carbon dioxide was used to chill the objective. The dry ice was packed into the space between the objective and the stage, above the upper cover glass. A piece of sheet asbestos was fitted over this to prevent the solid carbon dioxide from evaporating too quickly. A dissecting needle could easily be thrust through this layer of dry ice to tap the cover glass and prevent supercooling. The air space between the upper cover glass and the one on which the material was mounted gave adequate insulation against the low temperature induced by the presence of the solid carbon dioxide on the upper cover glass. Whenever this cover glass was broken, however, the mount froze immediately; therefore, no temperature readings were considered valid unless the cover glass remained unbroken throughout the freezing.

Exact determinations of temperature were not considered essential for this phase of the investigation. An alcohol-filled, low temperature thermometer reading to – 50° C. was inserted in the thermometer opening in the cold stage. This thermometer was calibrated by observing the temperature at which distilled water froze when placed between cover glasses in the same way as the plant tissues were mounted. This temperature was found to agree within 2° C. with the temperature as determined by immersing the thermometer in melting ice. There was less error when the temperature change was gradual than when it was rapid. Since the difference in temperatures was always greater than the experimental error, this method was considered sufficiently accurate. For more precise temperature readings it would be desirable to use a thermocouple instead of a thermometer.

To insure a uniform water content in experiments where wheat seeds were used, lots of 100 seeds were placed in 2-oz. bottles with a known volume of water. These bottles were then fitted securely with wooden wedges into holes in a large wooden wheel which was rotated slowly with a small electric motor. The rotation kept the grains constantly rolling over so as to bring about a uniform distribution of water. The seeds were weighed when air dry before the water was added to them, and then again after they had been rotated with the water for a given length of time. Some of the highest percentages of water determined may not have been accurate, since in some of these cases not all the water was absorbed.

For gross freezing, a General Electric water cooler was used. Alcohol, in which the bottles containing the seeds were suspended, was placed in the compartment designed for water, and the temperature was regulated in the usual manner. After treatment, the seeds were germinated in sterilized petri dishes containing layers of moist paper toweling.

Observations

For those studies involving microscopic observations, isolated cells of *Sedum acre* L., stamen hairs of *Zebrina pendula* Schnizl., and prothallia of *Polypodium aureum* L. with large irregular plastids were used.

Ice formation in cells of *Sedum acre* L.

The cells of the *Sedum* were obtained as follows: A leaf, split with a dissecting needle, was placed in a drop of paraffin oil on a slide and pressed gently under a cover glass. Then when the cover glass was lifted and the large fragments of the leaf were removed, isolated mesophyll cells, each surrounded by a thin film of cell sap released from the vacuoles of crushed cells, remained in the oil. These cells froze readily, but the thin film of sap froze more quickly than the cell contents and concealed the behavior of the latter.

Ice formation in *Zebrina* stamen hairs

The *Zebrina* stamen hairs were mounted directly in paraffin oil. The streaming, which at room temperature was fairly rapid, gradually became slower as the temperature dropped, and stopped completely at 0° to −2° C. In some instances Brownian movement of the granules began when streaming ceased; it persisted for a few seconds and then stopped. That this was not observed in all cases is probably attributable to the speed at which the temperature was lowered. No visible disintegration of the cells was evident after streaming had stopped. Mature cells froze when the temperature reached −7° or −8° C.; immature cells were more resistant, remaining unfrozen to a temperature of −17° C. These younger cells seemed to have a very high osmotic concentration which apparently decreases rapidly as the cells mature. These younger cells, moreover, showed larger granules and a greater concentration of anthocyanin.

As the freezing point was approached, it was necessary to tap the cover glass with a needle in order to prevent excessive undercooling. Formation of ice crystals always occurred first outside the field of vision, and as each cell froze, beginning at the end toward the base of the stamen hair, it inoculated the one next to it until in rapid sequence all of the cells in each hair had frozen. Each cell as it froze became filled with a closely packed mass of granular ice crystals, and was thereby rendered opaque. Differences in the freezing of the cell parts could not be observed. When cells frozen in this manner were thawed slowly, the cell sap escaped, since the plasma membrane had become more permeable, and the sap collected as droplets in the surrounding paraffin oil. When this occurred, the cells became shrunken and the cell walls very irregular. The cytoplasm assumed a very granular appearance and became stained purple by the vacuolar pigment. The nucleus was much distended and glassy in appearance and more conspicuous than

in the living cell. The appearance of cells after more rapid thawing was similar to that just described; however, in these cells, the cell retained more of its normal semipermeable character, since less of the cell sap escaped, and the cell walls had more nearly their usual contours.

Ice formation in *Polypodium* prothallia

The *Polypodium* cells, like those of *Zebrina,* showed excessive undercooling unless the cover glass was tapped with a needle. Ice formation took place in these cells in two ways; one of these may be described as "wave front advance" freezing, and the other as "single cell" freezing. In the first type, inoculation occurred outside of the field of the microscope, and from the first crystals seen, the ice front advanced in a series of localized waves, until the entire visible portion of the field had become frozen. The crystals formed under these conditions were compact and granular. In the "single cell" type of freezing, isolated individual cells froze here and there across the field until all the visible ones were frozen. In some of these, a slight quivering of the cell contents took place just as inoculation occurred, giving the impression that the protoplast had a jelly-like consistency. The ice crystals formed in some cells were compact and granular, like those of the "wave front advance" type. The crystals in other cells were needle-like and developed from one or more sides of the cell. These crystals elongated rapidly until they extended across the cell, giving the latter a striated appearance.

In all cases, the chloroplasts froze a few seconds after the other parts of the cell had been obscured by ice. This delay indicates the presence of a membrane around the plastid which may allow greater undercooling to occur. The plastid probably also has a higher osmotic concentration. Some differences seemed to exist between the time of freezing of the vacuolar sap and that of the cytoplasm, the latter freezing first; but the order of freezing could not be determined definitely with the apparatus used. The relative time of freezing of the nucleus remains uncertain.

When the temperature was raised, the crystals of ice melted gradually and formed drops of water. These drops fused and the protoplasts again became visible. The appearance of these protoplasts was comparable to that of the stamen hairs after thawing: the cytoplasm was granular and the outline of the plastids irregular. The plasma membrane likewise became more permeable, as evidenced by the droplets of cell sap accumulated in the oil outside. The cell walls retained their normal contours. No difference was observed between slowly and rapidly thawed cells.

Influence of sucrose as a protective agent

Experiments were performed with sections of the epidermis of red cabbage which had been kept in the cold room at 5° C. for several months and

presumably were in a hardened condition. Strips of epidermis were put into small test tubes, and the tubes were then immersed in an alcohol bath cooled to – 10° C. After the tubes had remained at this temperature for 10 minutes, chilled sucrose solutions of varying concentrations were poured gradually down the sides of the tubes. The sections were gradually brought to room temperature over a period of 2 hours and then examined under the microscope. Most of the cells showed some degree of plasmolysis, but there was no correlation between the extent of the plasmolysis and concentration of the thawing solution. When the sucrose solution was gradually replaced with distilled water, the protoplast disintegrated, the red pigment diffusing into the bathing solution. Even if the sucrose solution was diluted gradually over a period of an hour, in no case did the protoplast remain intact. This suggests that since the tubes in which the strips of epidermis were frozen were at room temperature when the experiment began, the cells became somewhat plasmolyzed by withdrawal of water during the first 2 or 3 minutes that the tubes were in the freezing bath. Then when they reached the temperature of the bath, or some critical point above this temperature, ice was quickly formed inside the protoplast and the cells retained the appearance they had at the time of freezing. The addition of the sucrose had no protective action in delaying deplasmolysis of the protoplast, but it did prevent the immediate disintegration of the cytoplasmic membranes which occurred in tissue thawed in air or water. The evidence suggests that the cells were dead when the sucrose solutions were added, and the "protective" action was purely mechanical.

To check these observations, the experiment was repeated. The test tubes used, however, were first chilled to the temperature of the bath before the strips of epidermis were introduced. The results were the same except that there was no pseudo-plasmolysis. The protoplasts were killed before any water was withdrawn from them.

Similar experiments were performed freezing the tissue in water and in solutions of sucrose ranging from 0.5 M to 4 M. At – 10° C., the water, 0.5, 1, and 2 M sucrose solutions appeared completely solidified. Ice crystals were formed in the two stronger solutions (3 and 4 M), but there was still a small amount of unfrozen solution present. If the solutions were cooled to the temperature of the bath before the epidermal strips were put into them, no plasmolysis occurred. But if the tissue remained in the solutions for a few minutes before being chilled, plasmolysis took place. When these sections were thawed gradually, and then the sucrose was replaced with distilled water, the protoplast swelled rapidly and the protoplasmic membranes disintegrated. Even when the dilution occurred gradually, the plasma membrane became disorganized and the cell sap diffused out into the bathing solution. In a few strips of epidermis, some two or three very

small cells deplasmolyzed completely and were apparently alive, but when they were plasmolyzed again, they did not survive. There is some doubt as to whether ice was actually formed in these cells, since they were very much smaller than the surrounding cells which did not survive, but even these smaller cells did not survive after the second plasmolysis.

ILJIN (9) found that similar strips of red cabbage epidermis were protected by thawing in sugar solutions. He suggests that the solution absorbs the water which is lost from the cells when the temperature is lowered and prevents its rapid imbibition by the protoplast when thawing takes place. And if the rate of expansion of the cytoplasm is more nearly equal to that of the cell wall, rupture of the plasma membranes and subsequent death of the cell does not occur. ILJIN's experiments may be criticized because the deplasmolysis which was used as the criterion of life was not carried to completion. That the protoplast would expand partially was considered sufficient proof that the cell was alive. The studies of CHAMBERS and HÖFLER (2) with isolated tonoplasts have shown that this membrane can be made to expand and contract with solutions of various concentrations even outside the cell and entirely free from protoplasm. CHAMBERS and HALE (3) describe frozen epidermal cells of onion bulb scales in which the cytoplasm and nucleus had distintegrated as a result of freezing, yet the tonoplast or vacuolar membrane remained intact and showed deplasmolysis on thawing. The evidence presented by ILJIN is not entirely convincing that the cells were still alive after being thawed in the sucrose solutions.

In the present work some of the strips were frozen in sucrose solutions in the cold stage. If the cells were strongly plasmolyzed before freezing took place, the temperature required for ice formation was much lower than if the cells were not plasmolyzed, which is to be expected in view of the higher osmotic concentration within these plasmolyzed cells, and perhaps because of greater undercooling in this condition. When the cells were very strongly plasmolyzed, ice was not formed at $-10°$ C.

In ILJIN's experiments the temperature was lowered much more slowly than in those here reported. The tissues were kept at some of the higher temperatures for as much as 24 hours. This not only would allow hardening to take place, which would make the cells more resistant to low temperature, but also allowed for plasmolysis which so increased the osmotic concentration of the cells that ice formation was prevented until a very low temperature was reached. This is in agreement with the findings of ÅKERMAN (1).

Experiments such as have just been described were performed with fern prothallia also. The results were comparable.

ICE FORMATION, WATER CONTENT, AND COLD RESISTANCE OF WHEAT

Wheat was chosen for the material to be studied because so many of the previous studies of cold resistance and hardiness have been made with wheat,

and because the seed coats do not interfere with rapid absorption of water. During the winter of 1935–1936 a local commercial grain was used for most of the tests, and the results were checked with Minhardi. During 1936–1937, Minhardi was used altogether, with the exception of one group of experiments for which a non-hardy wheat, Leap, was obtained from the Department of Plant Breeding.

The percentages of germination of the controls was above 95 per cent. in all the wheat used and the seeds were kept in a sealed jar in a cool room to prevent any marked changes either in water content or in vigor. The grains were hand selected for uniform size and were divided into lots containing 100 seeds each. The weight of these seeds before any water was added was considered the "air-dry" weight. The "oven-dry" weight was obtained after drying samples of seeds in a vacuum oven for 288 hours at 90° C. The water content of air-dry grain was determined at intervals for different lots of seed to check the amount of water they contained, so as to be sure that the grain used over a period of time had the same initial water content. Calculated on this oven-dry basis, the air-dry seed contained 10 per cent. of water. For those lots of seed to which water was added, the percentages were computed from the increase in weight of water over the air-dry weight, the latter being considered as 10 per cent. from the previous determinations. The lots of seeds were weighed, put into bottles and a known volume of water added from a burette. The bottles were then stoppered tightly, put into the wheel, and rotated for 48 to 96 hours. In some of the first experiments, this rotation was performed at 25° C., but since at that temperature, seeds containing more than 31 per cent. moisture sprouted, a temperature of 4° C. was used. At the end of the period of rotation, the seeds were weighed again and the average percentage of water in each lot of seed was calculated. Seeds of each class according to their water content were then exposed to a temperature of $-20°$ to $-25°$ C. for 5 hours. After treatment they were germinated at 25° C. Counts were made at the end of 36 to 72 hours. The wheat germinated very quickly, and only rarely did those seeds which failed to germinate after 72 hours germinate at all.

There is no essential difference between the shape of the curve of germination with the Minhardi wheat and that of the Leap. These results are shown on the graph (fig. 1).

Sections of the endosperm of the wheat kernels cut freehand with a razor were mounted in mineral oil in the cold stage. No freezing could be observed except in those endosperms which were saturated with water. Since the endosperm could not be used, the embryo was tried. One slice was cut from the tip of the embryo and discarded; the next slice was used. These slices were cut freehand as thin as possible. Most of the sections used were in parts only one cell thick; probably they were between 10 and 20

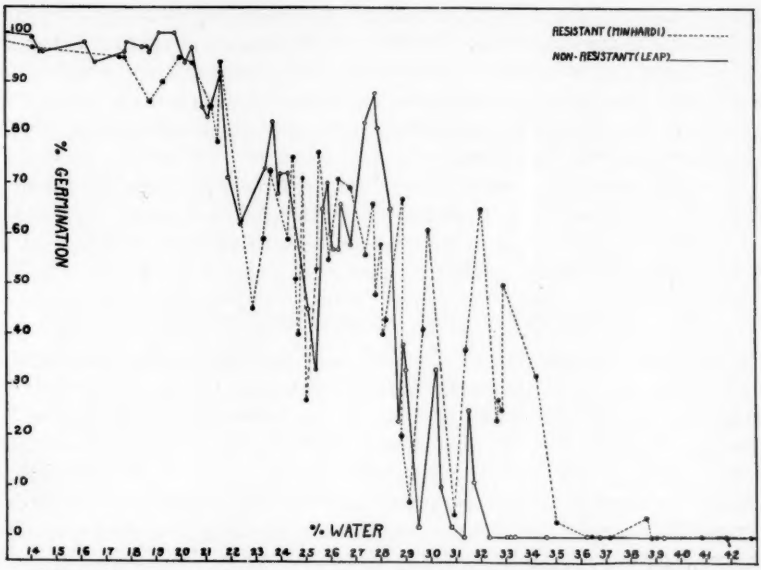

FIG. 1. Comparative behavior in germination of a resistant and a non-resistant variety of wheat at different moisture contents, after subjection to low temperatures (−20° to −25° C.) for 5 hours.

microns thick. No attempt was made here to find the temperature at which the seeds of different water contents froze. The purpose was to determine as quickly as possible whether seeds within certain water-content ranges would freeze or would not freeze. None of the seeds which contained 24 per cent. or less water would freeze even at temperatures down to −25° C. In the intermediate group (water content 27 to 28 per cent.) ice was observed in about half the sections but in the upper range (30 to 31 per cent.) all the sections examined showed ice formation. In some of the sections with 27 to 28 per cent. water, in which no ice was formed, apparently some ice formation took place in the intercellular spaces, but it was very difficult to distinguish between air and water in the intercellular spaces; it cannot be stated positively, therefore, whether or not freezing did occur in the intercellular spaces and not in the cells. When the temperature was lowered the cell contents became darker, perhaps because of some colloidal change or of a difference in the refractive index of the water at the low temperature. This darkening occurred regardless of ice formation. Finally, when the temperature became still lower, ice crystals appeared and the sections became opaque. The same precautions were taken to prevent excessive under cooling as were used with the other materials. To shorten the time for these

determinations, this freezing was done at temperatures lower than was necessary for ice formation. It was not as important to find the exact temperature at which ice was formed as it was to determine as rapidly as possible whether or not the embryos would show ice formation at any temperature. The results of the germination tests are shown on the graph. There was a correlation between the water content of the seeds, ice formation as observed in sections of the embryo, and their resistance to freezing. This relation between water content and resistance to freezing is in agreement with the findings of WHITCOMB and SHARP (**25**) for wheat, and of KIESSELBACH and RATCLIFF (**12**) for corn.

ICE FORMATION IN HARDENED AND NON-HARDENED WHEAT LEAVES

Plants of Minhardi wheat were grown in pots until they were about 6 inches high. Some of the pots were then placed in a refrigerator at 8° C. every night for ten nights and kept in the greenhouse during the day, while other pots remained in the greenhouse continuously. As far as possible, the soil moisture was kept the same for both sets of pots. ROSA found that cabbage plants exposed to sunlight and rather high temperatures during the day, and to temperatures close to freezing at night became hardened easily. The wheat plants hardened in this way were darker green, had thicker leaves, and were smaller than the actively growing plants. On one occasion, two pots containing 100 plants each, one set hardened and the other non-hardened, were left outside all night when the temperature reached −19° C. The hardened plants were not injured, while the non-hardened plants were so injured that only the roots survived. These plants subsequently produced more leaves when they were returned to the greenhouse. Neither the old nor the youngest leaves on the hardened plants were injured. When examined on the freezing microscope, the leaves from the non-hardened plants showed ice formation within the cells at −10° to −12° C., while the hardened leaves showed it first at −25° to −27° C. The cells were all killed when ice was formed, whether hardened or not. The hardiness was lost in three or four days if the plants were not placed in the refrigerator at night. When the pots were left in the greenhouse for three days, there was almost no difference in the temperature at which ice was formed in the leaves of previously hardened and non-hardened plants.

Discussion

Three hypotheses commmonly offered to explain the death of plants exposed to low temperatures are that this injury is due to drying, to a chemical agency, or to mechanical forces.

The chemical idea was first suggested by MÜLLER-THURGAU (**18, 19**) and strongly supported by MOLISCH (**17**) and GORKE (**8**), the theory being that

death resulted from some chemical action on the protoplasm effected by the removal of water from the protoplasm when ice is formed in the intercellular spaces. MOLISCH supposed the injury was caused by a toxic concentration of the cell sap, the increase in concentration being brought about by the dehydration. According to GORKE, the disintegration was thought to be caused by the precipitation of protective substances from the sap at their eutectic points leaving toxic materials to injure the protoplasm.

MOLISCH found that when filaments of *Spirogyra* were exposed to low temperatures, a crust of ice was formed around each of them. He believed that the water from which this ice was formed came from the interior of the cells, dehydrating them until the concentration of certain substances in the cell sap became so great as to injure the protoplasm. He could see no ice formed inside the cells and concluded, therefore, that death was caused entirely by this dehydrating effect. Our results with the *Sedum* cells have indicated that if a thin aqueous film is present around the cells, this layer of water is converted into ice when the freezing point is reached, and the behavior of the protoplast cannot easily be followed. Undoubtedly such a film was present around the algal filaments. This might explain why MOLISCH observed that the cells were dead after they had been thawed, even though he could see no ice inside them. WIEGAND (**26**) clearly demonstrated the presence of ice within the intercellular spaces of dormant buds, but these buds survived even though the cells adjacent to the ice were so dehydrated that the cell walls were compressed. According to the chemical theory the cells should have been killed by the high concentration of the cell sap. Several investigators have offered rather conclusive evidence that death on freezing is not caused by toxicity resulting from dehydration, and therefore increased concentration, of cell contents.

MAXIMOV (**15**), ILJIN (**9**), LEVITT and SCARTH (**13,** II) and CHANDLER and HILDRETH (**6**) support the theory that death at low temperatures is the result of mechanical injury either from ice crystals or from strong dehydration, rather than of chemical injury. ILJIN's suggestion that sometimes the killing is caused by the crushing of the protoplasm between the ice formed in the intercellular spaces and that formed in the vacuole is subject to serious criticism. He states that the concentration of the cell sap is less than that of the protoplasm and, therefore, ice must first be formed within the vacuole. LEVITT and SCARTH (**13,** I) seem to agree with this. They say: "When ice forms inside the cell, its site is the vacuole, not the protoplasm." In a later paper SCARTH and LEVITT (**23**) reiterate that intracellular ice formation, when it occurs, is confined to the sap vacuole. In their earlier paper they refer to CHAMBERS and HALE (**3**) as supporting this, but the latter, in describing the freezing in epidermal cells of the bulb scales of the red onion, cite cases and show photographs indicating ice in the cytoplasm external to

the tonoplast or vacuolar membrane when no ice was visible in the vacuole. In their summary CHAMBERS and HALE say: "The ice sometimes broke the outer membrane of the protoplast but the vacuole or tonoplast remains intact, in which case the tonoplast deplasmolyzes on thawing. Ice was not definitely seen to form within the vacuole but the general impression was obtained that it did so form in some cases."

Theoretical considerations indicate that ice formation would occur most readily outside the wall, followed in order by ice formation outside the outer protoplasmic membrane, then within the cytoplasm, and lastly within the vacuole. The osmotic concentration of the vacuole cannot be less but must be equal to or greater than that of the surrounding cytoplasm, for otherwise water would move to the outer, stronger solution. If the vacuolar membrane exerts any pressure whatever on the contained sap—and the surface tension alone of the membrane must be responsible for some pressure on the contents—this sap must be more concentrated than the bathing solution. If the vacuolar sap were less concentrated than that in the cytoplasm and froze at a higher temperature, it would be comparable to having a cell remaining turgid in a hypertonic solution and freezing at a higher temperature than the bathing solution. This situation could not obtain with a membrane permeable to water as the vacuolar membrane must be. Of course, although the vacuolar sap has a higher concentration and would therefore have a lower freezing point than the cytoplasm, the amount of freezing point lowering must be slight and this alone would not be important in preventing freezing. The combination of a membrane which would interfere with ice inoculation, together with this lower freezing point, may be responsible for ice formation in the cytoplasm first. Furthermore, since ice inoculation is likely to take place from the outside, this would favor ice formation in the cytoplasm before that in the vacuole even if they were at the same concentration. SCHANDER and SCHAFFNIT (**24**) describe ice formation as a thin layer just inside of the cell wall and between the protoplasm and the wall. The observations here reported, as well as those of SCHANDER and SCHAFFNIT, and of CHAMBERS and HALE (**3**), together with the theoretical considerations all indicate that ice formation takes place in the cytoplasm or between the cytoplasm and the wall before it does in the vacuole.

SCARTH and LEVITT (**23**) state that "Since at equilibrium 'suction tension,' and therefore the freezing point, must be the same in every part of a cell—vacuole, protoplasm, the cell wall—the osmotic value of the cells measured plasmolytically tells us the freezing point of the whole tissue." It is true that the suction tension of the cell as a whole and its wall are in equilibrium so that the freezing point at the surface of the cell is determined by the suction tension of the cell. That is, if cell *a* has zero suction tension, as it would have in distilled water, while cell *b* has a suction tension equivalent

to 0.5 M, ice would form at or in the wall of *a* at the freezing point of distilled water, while at the surface of cell *b* it would form only at or below the temperature necessary to freeze a 0.5 M solution, but in both cases the freezing point of the parts within the outer membrane may be very different and will be independent of the suction tension of the cell. For example, the suction tension of the nucleus, the vacuole, or a plastid will be determined not by the suction tension of the cell but by the osmotic concentration of their own immediate bathing media with which they are in equilibrium. As explained above if the vacuolar or plastid membranes exert any pressure on their contents, their osmotic concentrations must be higher and their freezing points lower than the cytoplasm within which they lie. Different parts may therefore have different freezing points and the osmotic concentration of the vacuole does not give an exact measure of the freezing point of the cytoplasm.

The low resistance to freezing observed by ILJIN (**10**) when the intercellular spaces of leaves are filled with water, as compared with normal turgid leaves or wilted leaves, presents a problem which would rarely occur under natural conditions. This free water would greatly favor ice formation in the cell. In those experiments in which ILJIN observed a protective action of sucrose or $CaCl_2$ when frozen tissues were thawed in the solutions, there is no proof that ice was formed within the cells and possibly in some cases not even in the intercellular spaces. He suggests that in most cases death occurs, not when ice formation takes place in the tissues but when the ice melts. The cells would, of course, be dehydrated by the formation of ice in the intercellular spaces. He suggests that when thawing occurs the walls and protoplast rapidly imbibe water and this rapid dilation or unequal swelling of different parts tears the protoplasm, thus resulting in death. If, on the other hand, this rapid or unequal swelling can be reduced by thawing in strong sugar solutions or those of $CaCl_2$ or similar salts of di- or trivalent cations which prevent rapid water absorption, killing is prevented; or the number of cells remaining alive is increased.

Two alternative explanations, however, may be offered. Possibly at the lower freezing temperature all of the cells were killed, but the strong salt or sugar solutions prevented complete deplasmolysis, and the cells therefore merely appeared alive. In the experiments with red cabbage here reported, frozen cells appeared alive if thawed in strong sugar solutions but on transference to dilute solutions or water they were found to be dead, whereas tissues which had not been killed by freezing retained their contents when placed in distilled water or dilute solutions. Another alternative is that, on slow freezing, ice had not formed in the cells but between them. Thawing in water or air may have supplied free water which would be available for ice formation in the cells which were undercooled or were at a temperature

low enough to form ice in the cell when water became available either from the melting intercellular ice or from the applied water or dilute solution. ILJIN gives no evidence that ice ever formed in cells that survived. In our own experiments killing occurred invariably when ice formed in the cells and, so far as was determined, only then.

Evidence from many sources, notably ÅKERMAN (**1**), ILJIN (**11**), and CHANDLER and HILDRETH (**6**), has demonstrated that a high osmotic concentration in cells increases their resistance to freezing. That the presence of solutes lowers the freezing point of the cell is obvious but it is not so obvious why there is often no direct relation between the increase in concentration and increased resistance. The failure to observe a direct relation between osmotic concentration and resistance to freezing is probably chiefly attributable to the fact that differences in rates of chilling, in permeability of the cell membrane to water, or differences in ratio of surface to volume of cell, and differences in amount of undercooling in part due to differences in readiness of ice inoculation across the outer membrane, may largely determine whether ice does or does not form in the cells. For example, a slow lowering of the temperature, high permeability to water, large surface relative to volume, and interference of the membrane to ice inoculation will greatly favor ice formation outside of the cells, thus bringing about a gradual increase in osmotic concentration inside the cell, with perhaps complete failure to form ice within the cells at the temperature to which the cells have been subjected. If the surface membrane interferes with inoculation across it, the fact that the ice outside the cell will have a lower vapor pressure than the undercooled solution inside may further favor outward movement of water, an increased osmotic concentration in the cell, and a lessened likelihood of intracellular ice formation. These several factors may explain the fact that a slight increase in osmotic concentration may be associated with greatly increased frost resistance.

Some investigators hold the opinion that hydrophilic colloids play a more important part than true solutes in increasing cold resistance, and suggest that these colloids by binding water prevent its loss from the cell and thus prevent killing by desiccation. ROSA (**22**) and NEWTON (**20, 21**) have consistently found increased bound water in hardened plants and suggest that the increase in bound water is responsible for the increased cold resistance. Many seem to think that the retention of water within the cell by the colloids is in itself significant. It is hard to imagine, however, that such bound water is any more available to the cell than if it were not there at all. The colloidal material itself, on the other hand, may serve as a protective colloid, perhaps preventing precipitation by its peptizing action; or better still, by reducing the free water and increasing the osmotic concentration would thus reduce the likelihood of ice formation.

CHRYSLER (7) found that in kelp stems the total amount of bound water increased as the amount of free water increased. If this holds for most plant materials tending to bind water (and there is a fair amount of evidence that it does), one would expect soaked seeds to contain more bound water than dry seeds, but the higher the water content of the seeds at the time of freezing the less is their resistance. With an increase in free water, on the other hand, even though there is an accompanying increase in bound water, there is an increased probability of ice formation and also a decreased resistance. This strongly points to lack of ice formation, not increased bound water, as responsible for resistance and that ice formation, not desiccation, is responsible for the injury. MEYER (16) actually found more bound water as well as more free water per gram of dry matter in the leaves of *Pinus rigida* in the summer, when the needles were not cold resistant, than in the winter when they were cold resistant.

Our experiments with hardened and non-hardened wheat leaves showed that free water was available for ice formation in the non-hardened leaves at $-10°$ to $-12°$ C. and they were killed at this temperature, but no ice could be observed in the section of hardened leaves until the temperature was lowered to $-25°$ to $-27°$ C. The resistance of dry wheat seed to ice formation and to killing, while those with more water formed ice and were killed, is further evidence that killing is not caused by desiccation or absorption of water after desiccation but to ice formation in the cells.

CHANDLER and HILDRETH (6) coated pollen of peach and *Amaryllis* with castor oil or cotton-seed oil, placed it on ice at $-15°$ to $-17°$ C., and water at 0° C. was slowly dropped on the pollen so that it froze as fast as it was added. If the oil was added to the dry pollen as it came from the anthers the freezing caused no injury, but if the pollen was first moistened no protective effect was noted. This is in agreement with our findings with dry and moistened seeds. It would seem that the oil had prevented absorption of water by the dry pollen and therefore no ice formed in the cells; but the moistened pollen had enough free water to allow for internal ice formation, and therefore killing, at the temperature used. The protective effect of the sugar solution is similar to that observed by ÅKERMAN and others, and is probably caused by an osmotic withdrawal of water and thus by prevention of ice formation within the cells.

From the observations here reported and the experiments of other workers the balance of evidence seems strongly in favor of the theory that death of plant tissues at freezing temperatures is brought about by mechanical injury due to ice formation within the cell. Anything that will decrease the amount of free water present will increase resistance to these low temperatures since ice is less likely to form within the cells. We have in no case found any cells to remain alive after ice once formed within them.

SCHANDER and SCHAFFNIT (**24**) and ÅKERMAN (**1**), however, cite cases in which they observed cells occasionally to remain alive after a small amount of ice was formed within them. By avoiding undercooling and cautiously lowering the temperature SCHANDER and SCHAFFNIT observed a thin layer of ice to form on the inner side of the cell wall at about – 5° C. If the tissue was then thawed the cells seemed alive as tested by plasmolysis with glycerine. If the temperature was further lowered the layer of ice grew thicker and the cells were killed. ÅKERMAN found that epidermal cells of red cabbage would withstand a small amount of internal ice formation without killing at – 5.2° C., but further lowering to – 7° or – 8° C. resulted in death. He found that any ice formation in cells of *Rhoeo* resulted in death. These cases of intracellular ice formation without killing were exceptional. They seem to demonstrate that ice can form in cells without killing but it is also conceivable that the ice formed between the wall and the cytoplasm, or that the injury was so slight that only the outer layer of cytoplasm was injured and that the tonoplast or vacuolar membrane was still intact and was responsible for the osmotic responses. CHAMBERS and HALE (**3**) observed ice formation in and disintegration of the cytoplasm and nucleus without destruction of the tonoplast membrane. It seems then that ice formation within the cytoplasm is almost certain to result in death and perhaps always so. Many claim that ice formation outside of the cell is usually responsible for the killing by freezing because of its dehydrating effect, but although one would expect such dehydration we know of no conclusive evidence that ice formation outside of the cell alone ever causes killing.

The observations of LEVITT and SCARTH (**13**, II) that cells hardened against freezing are much more permeable to water, strongly support the interpretation here given. As the temperature of the hardened cells is lowered the water would readily move through the surface membrane and form ice outside of the cell and therefore cause no injury. For the non-hardened cells free water would move out much more slowly and ice formation would be more likely to occur within the cell and result in its death. The conditions that reduce the likelihood of ice formation within the cell all seem to favor resistance to freezing. These are low amounts of free water within the cell, high osmotic concentration, high permeability of the membranes to water, small size of cell (high surface relative to volume), slow rate of temperature fall, and a membrane that favors undercooling by preventing inoculation across it.

Summary

1. A technique is described for direct microscopical observation of the freezing of living cells. When water or sap is present on the outer surface of cells the formation of an ice layer outside of the cells obscures what is

happening in the cells. Freezing in cells without this water film was observed when mounted in paraffin oil.

2. As the temperature of the cells of stamen hairs of *Zebrina pendula* was lowered, streaming became slower and finally ceased. Occasionally Brownian movement was visible for a few seconds after streaming stopped. Then ice formation took place at about $-7°$ C., appearing first in the basal cells and advancing across the field to the tip. Ice crystals were uniformly granular.

3. In the prothallia of *Polypodium aureum* granular crystals were usually produced, but occasionally needle like crystals were formed. Freezing occurred either cell by cell, or a wave of crystallization swept across the field. Insofar as could be determined the order of freezing agrees with the theoretical considerations; ice was formed first in the cytoplasm, then in the vacuole, and lastly in the plastids. The behavior of the nucleus could not be determined.

4. On thawing, the surface membrane was found to have lost its semipermeable character, allowing droplets of cell sap to collect in the mounting medium; the cytoplasm became granular and disintegrated; the nucleus assumed a glassy appearance.

5. Curves giving data on germination show that seeds of both the wheat varieties, Minhardi and Leap, were more resistant to freezing temperatures when the water content was low. Microscopic freezing tests of sections from the embryos of seeds containing varying amounts of water showed ice formation in seeds with high water and poor germination, and lack of ice in those with low water and good germination.

6. Leaves from plants of Minhardi wheat which had not been hardened showed ice formation at $-10°$ to $-12°$ C., whereas hardened leaves resisted ice formation down to $-25°$ C. Death always resulted if ice was formed in the cells.

7. Sucrose solutions did not protect strips of red cabbage epidermis from the injurious action of ice within the protoplast. If the cells were somewhat plasmolyzed by the solution before freezing began, a lower temperature was required for ice formation.

8. The evidence here presented and the observations of other workers strongly suggest that the death of plant tissues at freezing temperatures is caused by mechanical injury resulting from ice formation within the cells. Anything which will decrease the amount of free water within the cell at the time of freezing is likely to decrease the possibilities of ice formation within the cytoplasm and thus increase the resistance of the cell to freezing temperatures.

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TEMPERATURE EFFECTS UPON THE GROWTH OF EXCISED ROOT TIPS

GLADYS C. GALLIGAR

(WITH ONE FIGURE)

Introduction

Little work has been reported on the effect of different temperatures upon the growth of excised root tips in sterile nutrient solutions. WHITE (**6**) working with root tips of wheat found the optimum temperature for their growth to be between 26° and 27° C. This investigation was undertaken to determine the effect of different temperatures upon the growth behavior of excised root tips of dent corn, cotton, sunflower, and Burpee's Extra Early pea.

Materials and methods

Uniform lengths of 10 mm. were used throughout all series. Root tips of the varieties just mentioned, when ready for the experiment, were placed in five thermostats, maintained at temperatures of 10, 15, 20, 25 and 30° C. respectively, where they remained for a period of fourteen days. Five individuals for each temperature were used in each of five separate replicate series. Due to losses from contamination, data are reported for only twenty individuals at each temperature. Daily increments of growth were measured until the root tips passed the measurable stage, which occurred as early as the sixth day in sunflower at 20° C.

The procedure used to secure sterile root tips has been outlined in previous papers (**1, 2**). The nutrient solution was a modification of Pfeffer's formula to which dextrose and peptone were added.[1] Immediately after being made, the solution was measured into the flasks, which were closed with cotton plugs and autoclaved at 15 pounds pressure for 20 minutes.

Discussion

The results, presented in condensed form in tables I, II, III, IV, and V, demonstrate clearly the irregularity of growth even at uniform temperatures, and prove conclusively that the range in room temperature in previous studies (**1, 2**) cannot be employed to account for the irregular growth obtained.

[1] $Ca(No_3)_2$	2.0 gm.	KCL	0.25 gm.	Dextrose	2.0 per cent.
KH_2PO_4	0.5 gm.	M_gSO_4	0.5 gm.	Peptone	0.04 per cent.
KNO_3	0.5 gm.	$FeCl_3$	0.005 gm.	Distilled H_2O	6000 cc.

TABLE I

SAMPLE SERIES OF FIVE INDIVIDUALS EACH OF SUNFLOWER, COTTON, CORN, AND PEA SHOWING DAILY INCREMENTS IN MILLIMETERS OF LENGTH OF EACH INDIVIDUAL FOR FOURTEEN DAYS AT 10° C.

	Sunflower													
1	0	4	1	5	0	5	2	1	2	1	0	0	1	1
2	0	5	2	5	1	3	1	0	1	0	0	1	1	0
3	3	1	1	5	5	0	2	1	2	0	1	1	0	0
4	2	4	4	2	8	2	2	0	0	1	0	1	0	2
5	3	4	1	5	8	0	2	0	2	1	0	1	0	1
	Cotton													
1	1	0	0	1	2	0	1	1	1	0	0	2	0	1
2	0	1	0	0	1	1	0	0	0	0	0	0	0	0
3	1	0	0	1	0	0	0	0	0	0	0	0	0	0
4	1	1	0	1	0	0	0	1	0	0	0	0	0	0
5	0	0	1	0	1	0	1	0	0	0	0	1	0	0
	Corn													
1	2	2	3	3	3	1	1	2	1	0	0	1	0	0
2	2	4	3	5	2	4	2	4	4	2	2	1	2	3
3	0	4	4	5	2	3	5	2	1	2	1	0	0	0
4	2	1	1	2	4	2	0	3	2	3	2	1	2	2
5	2	3	4	2	1	1	2	2	1	1	0	1	2	3
	Burpee's Extra Early pea													
1	2	0	1	1	3	0	7	2	3	3	0	1	4	7
2	1	0	1	2	1	2	3	2	1	2	0	3	3	3
3	1	0	1	1	3	5	2	2	3	3	2	0	3	2
4	1	1	1	1	1	0	0	0	1	1	1	0	1	0
5	1	0	1	1	3	2	2	0	1	3	0	1	3	2

The root tips kept at 10° behaved similarly in some respects and quite dissimilarly in others. In no case was there an indication of laterals in sunflower, cotton, or pea, and only an occasional short lateral in corn. Characteristic papillae like those described previously (**1, 2**) developed on the sunflower root tips, but no laterals ever pushed through them. These papillae-like elevations were formed by secondary roots pushing outward against the epidermis. The proximal ends of the pea roots frequently became brownish red toward the end of the experiment; the same region of the cotton roots was often slightly blackened; anthocyanin developed to a considerable extent in corn; and sunflower roots retained their natural translucent appearance throughout the experimental period. On the whole, none of the roots were pigmented to any great degree at 10° C. It is of interest to note that an individual root tip of pea attained the greatest length of any root tip grown at 10° C., and that the root tips of pea were exceeded only by the root tips of corn in average total length and in dry weight at the end

of the fourteen-day period. These data indicate that the root tips of pea were more able to maintain growth activities under the given conditions than were those of cotton and sunflower. The increase in diameter of roots of corn and pea grown at 10° C. was markedly greater than that of the same species grown at 20° C., but less than when grown at 15, 25, and 35° C. The thickening was due to enlargement and proliferation of parenchyma cells of the cortex.

At 15° C. the root tips of pea did not branch; cotton and sunflower produced short laterals; and about ten per cent. of the individual root tips of corn produced twenty-five to forty laterals. Sunflower roots developed papillae, through some of which laterals made their way. The development of anthocyanin in corn was equal to that developed at 10° C.; the pea roots became pigmented at their proximal ends; cotton was somewhat more blackened than at 10° C.; and sunflower displayed no pigmentation. Corn root tips surpassed all others in total average length, in average number of

TABLE II

SAMPLE SERIES OF FIVE INDIVIDUALS EACH OF SUNFLOWER, COTTON, CORN, AND PEA SHOWING DAILY INCREMENTS IN MILLIMETERS OF LENGTH OF EACH INDIVIDUAL FOR FOURTEEN DAYS AT 15° C.

	Sunflower													
1	4	2	2	2	2	1	1	0	1	0	1	2	2	1
2	2	2	3	3	2	3	2	3	2	1	2	2	3	2
3	0	2	1	0	1	0	1	0	0	1	1	0	1	1
4	2	2	2	2	2	2	1	3	0	1	2	0	3	2
5	2	1	3	3	2	2	2	2	1	1	2	3	0	1
	Cotton													
1	3	0	0	3	2	0	2	1	1	1	1	1	0	1
2	1	2	1	1	2	0	1	2	2	1	1	0	2	1
3	2	1	1	3	2	2	2	0	1	0	2	2	3	1
4	1	1	2	0	1	2	0	0	2	1	2	2	1	0
5	3	0	2	3	2	2	1	1	2	2	0	2	2	1
	Corn													
1	3	2	3	4	5	2	3	3	2	3	2	1	2	3
2	2	1	2	0	8	2	4	4	5	8	2	1	3	2
3	4	4	5	4	2	2	2	2	2	1	3	2	2	2
4	3	4	3	2	1	7	3	4	6	5	2	2	3	2
5	2	3	4	3	2	4	3	5	3	5	4	2	3	4
	Burpee's Extra Early pea													
1	2	2	2	2	0	0	0	0	1	1	1	0	1	2
2	3	1	1	4	0	1	1	0	0	0	1	2	1	0
3	2	1	2	1	1	1	0	0	0	0	1	1	1	0
4	0	1	1	1	0	0	1	0	0	0	1	0	0	1
5	0	0	1	1	1	1	2	1	0	0	1	1	1	1

TABLE III

SAMPLE SERIES OF FIVE INDIVIDUALS EACH OF SUNFLOWER, COTTON, CORN, AND PEA SHOWING DAILY INCREMENTS IN MILLIMETERS OF LENGTH OF EACH INDIVIDUAL FOR FOURTEEN DAYS AT 20° C.

	Sunflower													
1	11	12	14	8	8	9								
2	5	10	8	7	9	9								
3	5	5	1	1	4	5								
4	4	6	3	4	5	4								
5	5	4	6	3	5	4								
	Cotton													
1	4	1	6	7	7	6	5	7						
2	3	1	2	4	3	3	2	2	1	3	1	1	2	1
3	4	2	5	1	1	1	2	5	4	1	2	1	2	1
4	0	2	1	2	2	3	4	1	0	2	2	1	1	2
5	3	2	4	1	2	2	2	3	3	1	2	2	1	2
	Corn													
1	1	1	2	6	6	7	5	5	6	7				
2	1	5	4	4	12	7	5	4	6	4				
3	4	5	4	5	1	1	2	7	5	4				
4	3	4	4	4	6	6	5	4	3	5				
5	4	3	3	5	7	6	4	3	3	5				
	Burpee's Extra Early pea													
1	2	3	3	0	2	0	1	0	0	0	1	0	0	0
2	3	1	1	0	2	0	0	0	0	1	0	0	1	0
3	3	0	1	5	1	0	1	0	0	0	0	1	0	0
4	2	1	0	0	2	0	0	0	0	0	0	2	0	1
5	2	2	1	1	0	0	0	1	0	0	1	0	0	1

branches, and in dry weight. Pea roots surpassed the roots of sunflower and cotton in dry weight, but surpassed cotton only in total length, and again as at 10° C. produced the longest single root tip.

At 20° C. corn and sunflower showed most branching, cotton produced a few short laterals, and pea none. There were a few individuals among corn and sunflower roots that had no laterals. These sharp individual variations from general tendencies were possibly due to genetic differences. Most of the laterals branched again within the fourteen-day period. Many of the corn roots developed anthocyanin. Cotton showed more blackening than at lower temperatures, many of the roots becoming completely pigmented. In total average length, dry weight, and number of branches corn exceeded all the roots, followed in order by sunflower, cotton, and pea.

At 25° C. corn and sunflower showed less branching than at 20° C., while cotton branched more. There was no pigmentation in corn, pea, and sunflower, but cotton became completely brown or black at the end of the

experimental period. The root tips of cotton excelled in average length of root and in average number of branches, while corn root tips were first in dry weight. This indicates that the protoplasm of the cotton roots was stimulated to excessive growth activity at this particular temperature with a resultant greater use of stored reserves in the older portion of the root.

At 35° C. only a few individuals of corn produced lateral roots, which were never more than five in number and always short and thickened. Pea and cottton became pigmented, but there was no coloration in sunflower or corn. At this temperature all the corn roots became much thickened due to hypertrophy of the cortex, which in some instances split away from the central cylinder, a condition found in earlier work (**1, 2**). Cotton and pea elongated on the average during the two week period about half again the length of the original fragment of root, sunflower about twice the original length, and corn three times the original length. In dry weight sunflower,

TABLE IV

SAMPLE SERIES OF FIVE INDIVIDUALS EACH OF SUNFLOWER, COTTON, CORN, AND PEA SHOWING DAILY INCREMENTS IN MILLIMETERS OF LENGTH FOR EACH INDIVIDUAL FOR FOURTEEN DAYS AT 25° C.

							Sunflower							
1	6	5	2	3	2	1	1	0	1	2	2	2	1	2
2	3	5	2	3	6	6	5	4	3	2	2	5	3	2
3	3	4	5	6	4	4	4	0	1	3	4	3	3	4
4	6	5	4	3	3	4	3	0	2	1	2	3	4	2
5	4	3	4	2	2	4	3	3	0	3	1	2	0	2
							Cotton							
1	3	4	4	4	10	13	12							
2	1	0	4	5	8	8	9	7						
3	3	2	7	8	6	6	8							
4	4	2	3	4	0	1	1	5	6	5	6	4	5	
5	4	5	5	4	11	14	9							
							Corn							
1	2	7	7	0	6	11	2	2	3	3				
2	1	4	4	7	6	8	3	3	2	2	4	3	2	
3	5	4	4	10	2	5	7	6	3					
4	3	5	5	8	4	5	10	4	2					
5	2	9	8	4	7	12	3	2	4					
							Burpee's Extra Early pea							
1	1	1	1	1	0	1	0	0	0	0	0	1	0	
2	2	1	1	0	1	0	0	0	1	0	0	1	0	0
3	2	2	1	2	1	5	0	0	0	0	0	0	0	0
4	2	1	1	1	1	0	0	1	0	0	1	0	0	0
5	1	2	1	1	1	1	0	0	0	1	0	0	0	1

TABLE V

SAMPLE SERIES OF FIVE INDIVIDUALS EACH OF SUNFLOWER, COTTON, CORN, AND PEA SHOWING DAILY INCREMENTS IN MILLIMETERS OF LENGTH FOR EACH INDIVIDUAL FOR A PERIOD OF FOURTEEN DAYS AT 35° C.

							Sunflower							
1	1	1	0	0	1	0	1	0	0	0	1	0	0	0
2	1	1	1	0	3	0	1	1	0	1	1	0	1	0
3	2	0	1	0	3	0	0	0	0	0	0	0	0	0
4	2	0	1	0	0	1	0	0	1	0	0	1	0	1
5	1	0	1	1	0	2	0	0	1	0	0	0	0	1
							Cotton							
1	3	1	1	0	1	0	0	0	0	1	1	0	0	0
2	4	0	1	0	1	2	0	0	0	0	0	0	1	3
3	4	1	0	1	0	0	0	0	0	0	0	1	0	0
4	2	0	1	1	1	0	0	0	0	1	0	0	1	0
5	3	1	1	1	0	0	0	0	1	0	0	0	1	1
							Corn							
1	1	2	1	2	1	1	1	1	0	0	0	1	0	0
2	0	2	4	1	1	2	1	0	0	0	1	2	1	1
3	2	2	3	1	0	1	3	0	1	1	0	0	1	1
4	3	3	3	2	3	1	0	0	1	1	0	0	1	1
5	2	2	1	2	3	0	1	0	0	0	1	1	1	1
							Burpee's Extra Early pea							
1	0	1	1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	1	0	1	1	0	0	0	1	0	0	0	0	0	0
5	1	0	1	0	1	1	0	0	0	0	0	0	0	0

cotton, and pea were approximately equal, while corn attained a dry weight of nine to ten times that of the other three.

The behavior of the root tips of pea was unusually interesting. Average total length and average dry weight indicate that these roots perform best at a temperature of 10° C. At 15° C. they also performed better than at the higher temperatures, 20° and 25° C., but the dry weight at 15° C. was 50 per cent. greater than it was at 20 and 25° C. Since the root tips used in this experiment grew better under the given conditions at 10° C., it cannot be said in general that 10° C. is the optimal temperature for the growth of pea roots. It is the optimal temperature for the prescribed conditions for a fourteen day period. It is possible that the lower temperature inhibited the absorption of some constituent in the medium, which, when absorbed at higher temperatures exercised an inhibitory effect upon the growth activities of the root tips. On the other hand the better growth may have been caused by an internal response to the lower temperature. There

TABLE VI

TABLE VI

SUMMARY OF DATA ON 600 ROOT TIPS CUT AT ORIGINAL LENGTHS OF 10 MILLIMETERS AND ALLOWED TO GROW 14 DAYS AT DIFFERENT TEMPERATURES IN THE BASIC SOLUTION

TEMPERATURE ° C.	SPECIES	AVERAGE DAILY INCREMENT OF LENGTH OF 20 ROOT TIPS FOR 1ST 10 DAYS										AV. TOTAL LENGTH AT END OF 14 DAYS	RANGE IN LENGTHS OF INDIVIDUAL AT END OF 14 DAYS	AV. MULTIPLICATION OF THE ORIGINAL LENGTH IN 14 DAYS	AVERAGE NO. OF LATERAL ROOTS	RANGE IN NO. OF LATERAL ROOTS AMONG INDIVIDUALS	AV. DRY WT. PER 10 ROOTS
		1	2	3	4	5	6	7	8	9	10						
		mm.	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*				*gm.*
10	Corn	1.53	2.71	2.73	3.77	3.75	2.50	2.03	2.58	2.24	2.00	39.	20–45	3.9	0.3	0–4	0.125
	Pea	1.21	0.23	1.00	1.24	2.00	2.10	3.00	1.00	2.56	2.00	36.	17–65	3.6	0.0	0	0.035
	Sunflower	1.29	3.51	1.73	4.20	3.50	2.51	1.26	0.20	0.78	0.20	34.	16–49	3.4	0.0	0	0.027
	Cotton	0.70	0.20	0.00	0.53	0.59	0.34	0.00	0.23	0.20	0.27	14.	10–23	1.4	0.0	0	0.022
15	Corn	3.00	2.70	2.67	2.50	4.55	1.75	4.00	3.00	3.10	5.20	41.	24–70	4.1	5.0	0–40	0.114
	Pea	1.70	1.52	1.23	2.01	0.30	0.20	0.51	0.00	0.00	0.20	29.	16–76	2.9	0.0	0	0.032
	Sunflower	2.04	2.30	2.12	1.70	1.75	1.56	1.23	1.50	0.70	0.20	32.	15–60	3.2	2.0	0–10	0.025
	Cotton	1.70	1.05	1.11	1.72	1.67	1.10	1.69	0.47	0.73	0.20	13.	10–30	1.3	0.62	0–8	0.030
20	Corn	2.21	3.54	3.70	5.21	4.73	7.02	4.70	5.20	4.08	4.58	109.	53–152	10.9	41.0	0–75	0.200
	Sunflower	6.24	4.71	6.57	5.00	6.52						57.	23–110	5.7	11.0	0–43	0.034
	Cotton	2.77	1.54	3.20	3.60	3.41	3.22	3.74	2.79	3.07	3.24	43.	21–71	4.3	9.0	3–14	0.028
	Pea	2.52	1.26	1.24	0.20	2.74	0.00	0.50	0.03	0.00	0.22	30.	24–95	3.0	0.0	0	0.023
25	Corn	7.53	5.00	5.50	4.50	4.57	6.73	1.75	1.23	1.26	1.25	57.	32–118	5.7	14.0	0–66	0.090
	Cotton	2.70	2.00	4.59	5.20	7.54	7.00	7.57				98.	30–136	9.8	30.0	14–45	0.033
	Sunflower	5.10	4.72	3.26	3.74	3.67	3.58	3.90	1.00	1.56	2.30	44.	17–67	4.4	4.0	0–15	0.028
	Pea	1.78	1.20	1.07	1.06	0.73	1.58	0.00	0.20	0.20	0.02	29.	16–76	2.9	0.0	0	0.021
35	Corn	1.51	2.22	3.06	1.21	1.03	1.26	1.05	0.07	1.00	0.52	30.	23–37	3.0	0.46	0–5	0.076
	Sunflower	1.56	0.50	0.73	0.00	1.70	0.21	0.53	0.30	0.00	0.56	19.	12–35	1.9	0.0	0	0.008
	Cotton	2.21	1.01	0.50	0.53	0.76	0.54	0.00	0.00	0.00	0.56	15.	13–20	1.5	0.0	0	0.006
	Pea	0.00	0.21	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13.	11–15	1.3	0.0	0	0.007

TABLE VII

SUMMARY OF THE DATA ORGANIZED TO SHOW THE BEHAVIOR OF EACH SPECIES

Species	Temperature	Average daily increment of length of 20 root tips for 1st 10 days										Av. total length at end of 14 days	Range in lengths of individual at end of 14 days	Av. multiplication of the original length in 14 days	Average no. of lateral roots	Range in no. of lateral roots among individuals	Av. dry wt. per 10 roots
		1	2	3	4	5	6	7	8	9	10						
	° C.	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*				*gm.*
Corn	10	1.53	2.71	2.73	2.77	3.73	2.50	2.03	2.58	2.24	2.00	39.	20–45	3.9	0.3	0–4	0.125
	15	3.00	2.70	2.67	2.50	4.55	1.73	4.00	3.00	3.10	5.20	41.	24–70	4.1	5.0	0–40	0.114
	20	2.21	3.54	2.70	5.21	4.73	7.02	4.70	5.20	4.08	4.58	109.	53–152	10.9	41.0	0–75	0.200
	25	7.53	5.00	5.50	4.50	4.57	6.73	1.75	1.23	1.26	1.25	57.	32–118	5.7	14.0	0–66	0.090
	35	1.51	2.22	3.06	1.21	1.03	1.26	1.05	0.07	1.00	0.52	30.	23–37	3.0	0.46	0–5	0.078
Sun-flower	10	1.29	3.51	1.73	4.20	3.50	2.51	1.26	0.20	0.78	0.20	34.	16–49	3.4	0.0	0	0.027
	15	2.04	2.30	2.12	1.70	1.75	1.56	1.23	1.50	0.70	0.20	32.	15–60	3.2	2.0	0–10	0.025
	20	6.24	4.71	6.57	5.00	6.52						57.	23–110	5.7	11.0	0–43	0.034
	25	5.10	4.72	3.26	3.74	3.67	3.58	3.90	1.00	1.56	2.30	44.	17–67	4.4	4.0	0–15	0.028
	35	1.56	0.50	0.73	0.00	1.70	0.21	0.53	0.30	0.00	0.56	19.	12–35	1.9	0.0	0	0.008
Cotton	10	0.70	0.20	0.00	0.53	0.59	0.54	0.00	0.23	0.20	0.27	14.	10–23	1.4	0.0	0	0.022
	15	1.70	1.05	1.11	1.72	1.67	1.10	1.69	0.47	0.73	0.20	13.	10–30	1.3	0.62	0–8	0.030
	20	2.77	1.54	3.20	3.60	3.41	3.22	3.74	2.79	3.07	5.24	43.	21–71	4.3	9.0	3–14	0.028
	25	2.70	2.00	4.59	5.20	7.54	7.00	7.57				98.	30–136	9.8	30.0	14–45	0.033
	35	2.21	1.01	0.50	0.53	0.76	0.54	0.00	0.00	0.00	0.56	15.	13–20	1.5	0.0	0	0.006
Pea	10	1.21	0.23	1.00	1.24	2.00	2.10	3.00	1.00	2.56	2.00	36.	17–65	3.6	0.0	0	0.035
	15	1.70	1.52	1.23	2.01	0.30	0.20	0.51	0.00	0.00	0.20	29.	16–76	2.9	0.0	0	0.032
	20	2.52	1.26	1.24	0.20	2.74	0.00	0.50	0.03	0.00	0.22	30.	24–95	3.0	0.0	0	0.023
	25	1.78	1.20	1.07	1.06	0.73	1.58	0.00	0.20	0.20	0.02	29.	16–76	2.9	0.0	0	0.021
	35	0.00	0.21	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13.	11–15	1.3	0.0	0	0.007

was little growth at 35° C. The roots exhibited marked increase in diameter at 10 and 25° C. owing to enlargement of the cortical cells. No lateral roots developed at any temperature used.

Root tips of corn gave their best performance at 20° C., which is 12° C. lower than the optimum temperature found by LEHENBAUER (3) for the growth of young shoots of corn. LEHENBAUER pointed out that the optimum temperature for growth under the conditions of his experiments shifted somewhat as the time factor was varied.

Sunflower also behaved best at 20° C. At 10° and 25° C. no lateral roots appeared within the fourteen day period, but the characteristic papillae, which frequently appeared in sunflower, and from which laterals later protruded, occurred on many individuals at both temperatures, but they occurred in greater numbers on those at 10° C. than on those at 35° C. In no case did a moniliform root develop, which was probably due to the fact that the time period used was too short for such a phenomenon to occur. In many individuals at 35° C. the proximal ends of the root tips became unduly thickened owing to a proliferation of cortical parenchyma and to excessive enlargement of cells of the same tissue. It is of interest to note that in final average total length and in average dry weight the root tips at 10° C. slightly exceeded those at 15° C. This observation may possibly have some correlation with the behavior of colloidal substances in the protoplasm, which are known to manifest peculiar reactions in the neighborhood of 15° C., which differ from those occurring at higher and lower temperatures.

Cotton showed optimal average total length and optimal average dry weight at 25° C. Compared with cotton roots grown at room temperature as long as growth would continue, the 25° cultures averaged more than 50

FIG. 1. Root tips maintained at constant temperatures for 14 days. Reading from left to right are four groups of root tips: cotton, corn, sunflower, and pea respectively. In each group the tips in the top row were maintained at 10° C., in the second row at 15° C., in the third row at 20° C., in the fourth row at 25° C., and in the bottom row at 35° C.

per cent. longer, and approximately 25 per cent. greater in dry weight. There was scarcely any growth in length at 10° and 35° C. An examination of the dry weight figures for these temperatures indicate that the root tips at 35° C. drew heavily upon the reserves in the older part of the root in order to remain alive.

Figure 1 presents a few samples of each species of root tip from each temperature used, and shows at a glance general trends in the behavior of all the root tips used at the various temperatures.

Summary

1. The optimal temperature for excised pea roots was 10° C., for sunflower and corn 20° C., and for cotton 25° C.

2. Few or no lateral roots developed at temperatures of 10° and 35° C.

3. Temperatures of 25° and 35° C. inhibited the formation of anthocyanin in corn.

4. Temperatures of 10° and 15° C. retarded pigmentation in cotton.

5. Marked distension in diameter occurred in roots of pea at 10° and 25° C. and in corn and sunflower roots at 35° C.

6. 35° C. was definitely beyond the range of optimal temperature for growth of root tips of corn, pea, sunflower, and cotton.

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CARBOHYDRATES OF BEAN PLANTS AFTER TREATMENT WITH INDOLE-3-ACETIC ACID

TAYLOR R. ALEXANDER

(WITH SEVEN FIGURES)

Introduction

During recent years, a number of histological and morphological studies of the tumor-growth responses of bean plants to indole-3-acetic acid have been published (**1, 4, 6**). One investigation of etiolated bean plants indicated that indole-3-acetic acid (heteroauxin) affects the translocation of carbohydrate and nitrogenous material (**7**). The general stimulating effect of indole-3-acetic acid on the synthesis of solid matter by bean plants is known (**6**). WENT and THIMANN (**10**) state that in the *Avena* coleoptile there are deposited on the cell wall large amounts of cellulose, pectin, and hemicelluloses, but that there is no direct relationship between the amount of these substances and the amount of auxin applied. They state further that the action of auxin in these processes is indirect.

Although one would expect the rates of respiration to differ between indoleacetic-acid treated and control plants, reports have not confirmed this assumption (**7**).

The present investigation concerns some of the carbohydrate changes which are related to the structural and weight differences previously observed in plants treated after decapitation.

Investigation

MATERIALS AND TREATMENT

The plant used was the Dwarf Red Kidney bean, *Phaseolus vulgaris*. The seeds were selected for uniformity in size and in appearance. Two crops, of approximately 1,000 plants each, were grown, crop 1, Feb. 20 to Mar. 10, and crop 2, Feb. 22 to Mar. 12. The plants were grown in quartz sand in 5-inch clay pots. A more uniform population was obtained, and the shedding of the seed coats was facilitated, when the seeds were planted with the raphe facing downward, the seed inclined at an angle of 45°. After planting, the pots were watered with warm tap water. The temperature of the greenhouse fluctuated from 58° to 90°F. during the course of the experiment, but averaged about 75°F. The relative humidity varied from 30 to 80 per cent., but was generally below 50 per cent. Crop 1 germinated during very cloudy weather, while crop 2 came up under sunshine of high light intensity. After Feb. 27, the sun was bright except for three days.

Six days after planting, the plants had emerged through the surface of the sand. The plants were then culled, leaving a very uniform crop, and the pots were watered with warm nutrient solution (**8**). The nutrient treatment was repeated every fourth day, the sand being kept moist with tap water between the additions of nutrients.

Ten days after planting, the plants were about nine inches high, and the second internode was one to two inches long. The crops were divided into three equal groups, of approximately 300 plants each—initial controls, final controls, and treated plants. The second internode was cut off 2 cm. above the point of divergence of the primary leaves. The initial control group was then harvested. The cut surface of the final control group was covered with 3 to 4 $mm.^3$ of lanolin. The third group was treated with the same amount of lanolin in which indole-3-acetic acid had been thoroughly mixed. The two per cent. paste was made by melting 5 gm. of anhydrous lanolin, and vigorously stirring into it 100 mg. of indole-3-acetic acid (Merck). Following treatment, the treated and untreated plants were placed in alternate rows, so that an equal number of both were exposed to nearly the same enviromental factors. In all lots axillary shoots were removed as soon as they appeared as small buds.

Seven days after treatment, the final controls and treated plants were harvested and divided into the following samples: upper stem end, lower stem end, primary leaves, stem, and roots (see fig. 1). The roots were freed of sand by dipping them momentarily into a saturated salt solution and then washing thoroughly with distilled water. The initial controls had been harvested in a like manner.

Physical measurements

Fresh weights of all samples were determined by use of a torsion balance. The tissue was then placed in a well-ventilated drying oven, and dried to constant weight at 80°C. The oven-dried samples were placed in dry Mason jars, sealed, and allowed to cool. In this way moisture adsorption was minimized, and later, dry weights were determined. A torsion balance, sensitive to 0.01 gm., was used for the large, and an analytical balfor the small samples.

Before the stem ends could be ground, the lanolin had to be removed. This was accomplished by a one-minute extraction with petrol ether. Alpha-naphthol tests on the ether after extraction showed a very faint carbohydrate test. The ends were redried, and a new dry weight was taken from which to calculate the data.

Chemical methods

The small samples were ground by hand with mortar and pestle until the powder would pass an 80-mesh screen. The large samples were finely

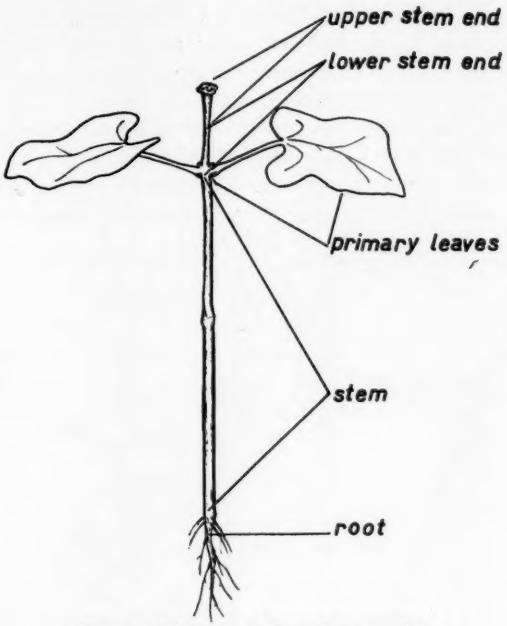

FIG. 1. Division of plant into samples.

ground in a coffee mill. Then a 5- to 10-gram portion was ground to 80-mesh fineness by the use of a ball mill. All samples were redried at 80°C., and placed in a desiccator.

The analyses were run on duplicate 1-gm. samples for crop 1 and single samples for crop 2. These were placed in weighed filter papers, and extracted with hot 75 per cent. alcohol in Soxhlet extractors on a sand bath. Each extraction was checked for completeness by the alpha-naphthol test. At the end of the first hour the alcohol in the receiving flasks was replaced by a fresh quantity to prevent overheating of the sugars, as suggested by LOOMIS and SHULL (**5**).

The alcohol-soluble sugar extract was prepared and analyzed by the ceric sulphate method of HASSID (**2, 3**), using Setopaline C as the indicator. Sucrose was also determined by this method, after using invertase for inversion.

After the sugar extraction, the net residue was weighed. The sample was then ground to 100-mesh fineness, redried, and an aliquot was weighed out for the determination of polysaccharides. Starch and dextrin, water-soluble acid-hydrolyzable, and insoluble acid-hydrolyzable substances were hydrolyzed and calculated according to the procedure of LOOMIS and SHULL.

Saliva was used for the starch and dextrin hydrolyses, and 1 + 20 HCl was used for the other two hydrolyses. The above-mentioned carbohydrate fractions were determined as reducing sugars on the cleared and neutralized extracts by the ceric sulphate method.

The solution containing the acid-hydrolyzable substances showed considerable color, and determinations made upon duplicate colored samples did not check with sufficient accuracy. The samples were, therefore, decolorized by treating a 25-cc. aliquot of the neutralized solution with 0.3 gm.

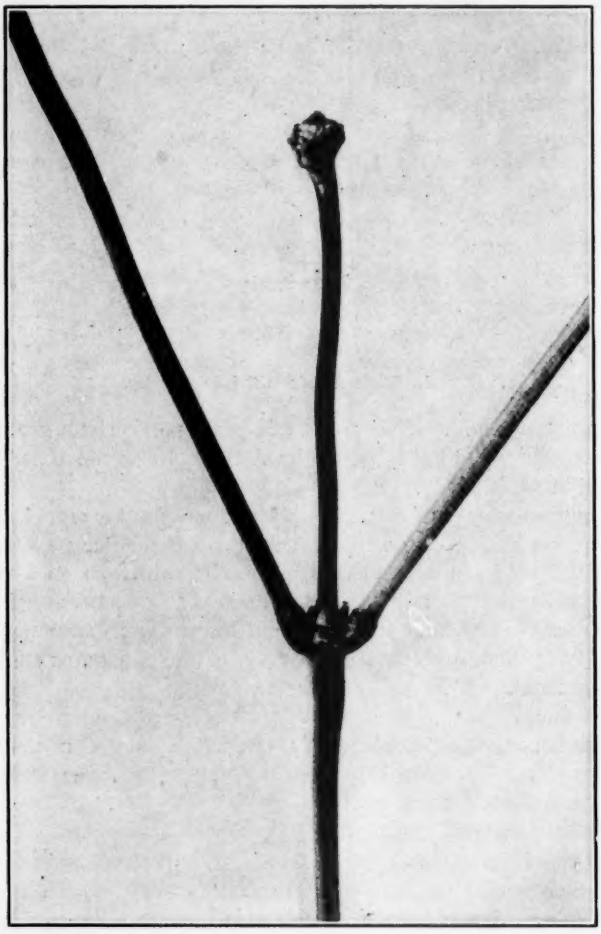

FIG. 2. Treated stem end at time of harvest.

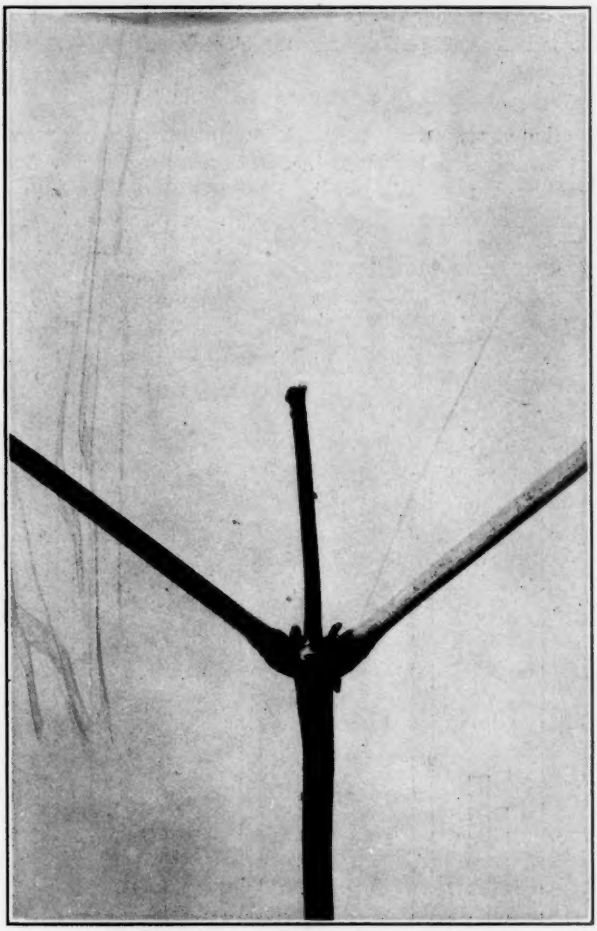

FIG. 3. Control stem end at time of harvest.

of charcoal for 15 minutes, after which it was filtered with suction. The sample was then brought up to a 100-cc. volume, and aliquots of this cleared solution were used for the titrations. With this modification, duplicate determinations checked very closely.

Results and discussion

MORPHOLOGICAL OBSERVATIONS

The response of the plants to the heteroauxin treatment was identical to that reported previously (**4, 6**) (see figs. 2 and 3). Tumors and roots

were produced, the former shown in figure 2. The final control plants produced a large number of small calluses at the cut surface.

Weight differences

Examination of table I shows that the upper stem end responded to the indoleacetic acid treatment by an increase of approximately five times as much dry weight as the final control. The entire stem end, both upper and lower portions, shows about a 300 per cent. gain (see figs. 4 and 5). The treated stem ends of crop 2 gained more than those of crop 1.

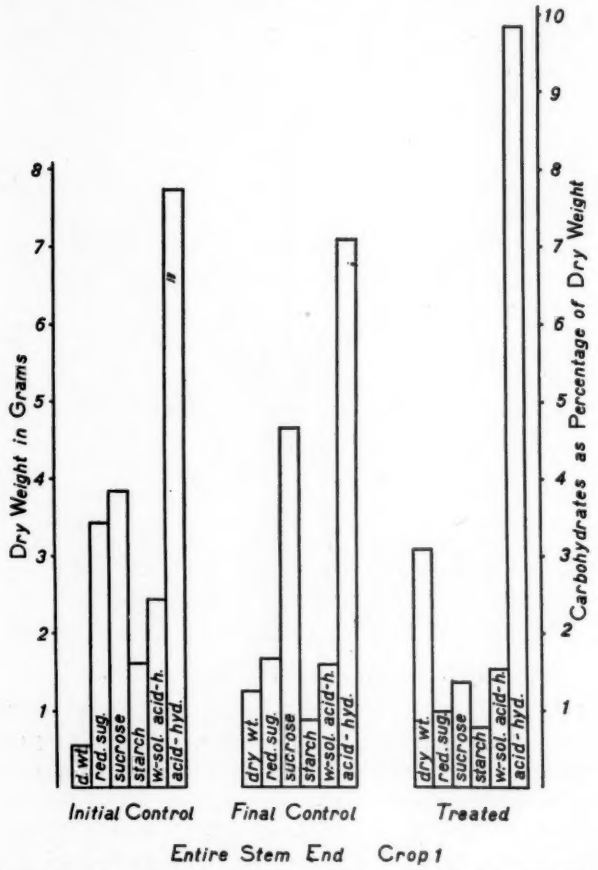

Fig. 4. Comparison of dry weight and chemical composition (data based on grams per 100 plants).

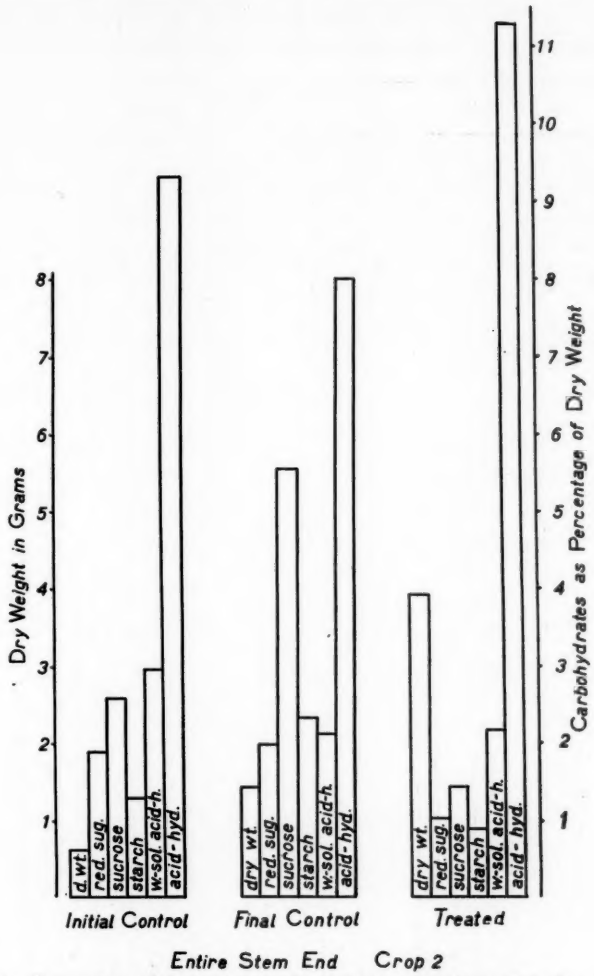

FIG. 5. Comparison of dry weight and chemical composition (data based on grams per 100 plants).

With regard to the entire plant, the total weight of the final controls is heavier in both crops (figs. 6 and 7). There is a greater difference between the dry weight of the final control and treated plants in crop 2 than in crop 1. The primary leaves account for most of the differences in total dry weight, but it is not a matter of increase in leaf area following heteroauxin application (**6**).

TABLE I

DRY WEIGHTS (FIGURES REPRESENT GRAMS OF DRY WEIGHT PER 100 PLANTS)

REGION	INITIAL CONTROL	FINAL CONTROL	TREATED
Crop 1			
Upper stem end	0.16	0.40	1.87
Lower stem end	0.39	0.86	1.44
Primary leaves	20.22	40.60	35.92
Stem	10.93	19.00	18.17
Roots	7.21	12.00	11.61
Total	38.91	72.86	69.01
Crop 2			
Upper stem end	0.17	0.42	2.28
Lower stem end	0.44	1.00	1.64
Primary leaves	18.17	45.83	39.18
Stem	8.64	19.22	17.68
Roots	6.92	13.43	12.79
Total	34.34	79.90	73.57

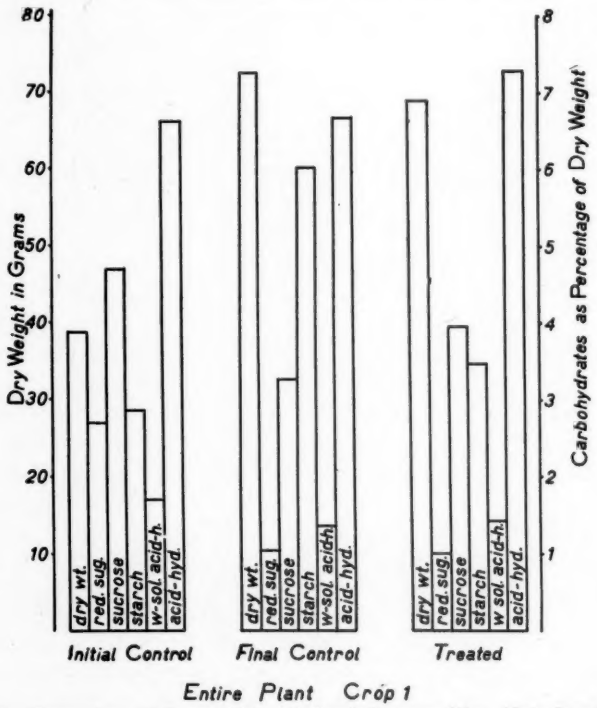

FIG. 6. Comparison of dry weight and chemical composition (data based on grams per 100 plants).

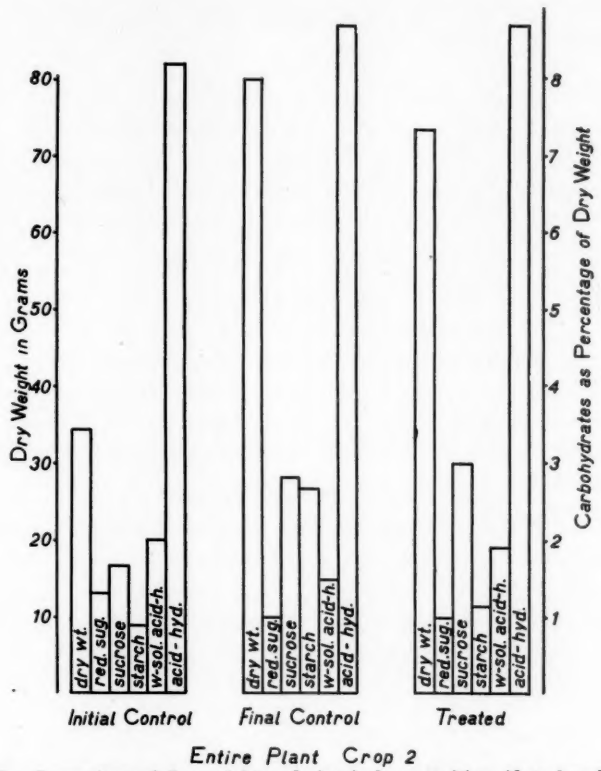

FIG. 7. Comparison of dry weight and chemical composition (data based on grams per 100 plants).

The increase in weight seems to indicate several things. First, small changes in the environment cause quantitative differences in the response to indoleacetic acid treatment. As indicated before, crop 1 was grown under lower light intensity than crop 2, especially during the early stages of development; and, therefore, crop 1 was etiolated in appearance as compared to crop 2. The effect is evidenced in the amount of dry matter synthesized both in the entire plant and in the stem end. Second, the decrease in dry weight of the primary leaves, stems, and roots of the treated plants indicates a movement of material to the region where heteroauxin had been applied (**6**). Third, the loss in weight of these parts is greater than the gain made by the stem ends, so it seems highly probable that a part of the translocated substances was respired in the very active tissues of the tumor. Fourth, crops 1 and 2 have a 5.3 and 7.9 per cent. difference in weight,

respectively, in favor of the final control plants; the stem ends of crop 1 are the lighter. In other words, the stem end of crop 2 gained 17.8 per cent. more than those of crop 1, and, at the same time, the primary leaves, stems, and roots of the treated plants of crop 2 lost 33 per cent. more than those of crop 1. The assumption that the treated plants were capable of and did synthesize at least as much dry matter as the final controls, and that there really was a loss in the treated plants, is based on the work of MITCHELL and HAMNER (6). They found that plants treated in a similar manner with two per cent. indoleacetic acid paste had gained, at the end of six days, in dry weight over the controls, if the axillary shoots were not removed. At the same time, a large tumor was formed. The additional leaf area evidently supplied enough material for the tumor growth and in addition the heteroauxin stimulated the photosynthetic process. Points three and four suggest to the writer that, under the conditions of the experiment, respiration is accelerated in the treated stem end to furnish energy for the excessive growth and translocation processes.

CHEMICAL COMPOSITION

Figures 4, 5, 6 and 7, and table II give the chemical composition of the various plant parts. The upper stem end shows, in general, smaller percentages of the more complex fractions than the lower stem end. This indicates that there is another substance or substances present in large amount. These substances might be nitrogenous in nature, but belong most likely to the structural polysaccharides. The chemical nature of the entire stem end of the treated plants of both crops is very similar. The main difference is a greater percentage of water-soluble acid-hydrolyzable and insoluble acid-hydrolyzable material in crop 2. This may be associated with differences in light intensity. The final control plants follow the same trend with respect to the last two groups of compounds mentioned; in addition they are high in sucrose.

The higher percentage of carbohydrates in the final controls of crop 2 suggests that photosynthetic activity was favored by the more intense light. As compared to the treated stem ends, the metabolically active carbohydrates are higher instead of the insoluble acid-hydrolyzable materials. The difference in the reducing sugar and sucrose content of the initial controls of the two crops can be partially explained by the fact that crop 1 was harvested late in the afternoon, while crop 2 was harvested late at night when one would expect condensation to have taken place and respiratory losses to have occurred. Glucose is such a variable quantity that no great stress should be laid on its variation for interpretation of studies of this kind.

The entire plants of both crops have a chemical composition that is com-

TABLE II

CHEMICAL COMPOSITION (DATA AS PERCENTAGE OF DRY WEIGHT PER 100 PLANTS)

REGION	REDUCING SUGARS			SUCROSE			STARCH			WATER-SOLUBLE ACID-HYDROLYZABLE			ACID-HYDROLYZABLE		
	INITIAL CONTROL	FINAL CONTROL	TREATED	INITIAL CONTROL	FINAL CONTROL	TREATED	INITIAL CONTROL	FINAL CONTROL	TREATED	INITIAL CONTROL	FINAL CONTROL	TREATED	INITIAL CONTROL	FINAL CONTROL	TREATED
Crop 1															
Upper stem end	6.18	1.60	1.07	3.88	5.50	1.29	1.69	0.80	0.87	3.06	1.57	1.51	6.50	5.80	8.90
Lower stem end	2.28	1.76	0.88	3.82	4.29	1.45	1.62	0.92	0.75	2.16	1.62	1.56	8.24	7.70	11.15
Primary leaves	2.64	0.82	0.96	5.47	2.85	3.42	4.79	8.22	5.54	1.94	1.31	1.44	4.76	5.08	4.99
Stems	3.20	1.58	1.29	4.98	5.33	3.58	0.69	4.90	1.56	1.30	1.22	1.30	8.16	8.02	9.23
Roots	1.59	0.75	0.60	2.14	1.27	0.70	0.76	0.75	0.76	1.66	1.70	1.59	9.38	10.03	10.50
Entire plant	2.69	1.03	1.00	4.71	3.25	3.95	2.83	6.04	3.46	1.71	1.36	1.43	6.61	6.68	7.28
Crop 2															
Upper stem end	2.82	2.86	1.01	1.11	7.61	1.01	1.12	3.48	0.78	3.62	2.69	2.15	8.36	6.36	10.18
Lower stem end	1.57	1.62	1.04	2.46	4.72	2.01	1.36	1.84	1.01	2.73	1.88	2.17	9.65	8.70	13.17
Primary leaves	1.16	1.05	0.99	1.69	3.00	4.12	0.51	1.33	1.02	2.28	1.38	1.86	6.33	7.85	6.87
Stems	1.74	1.15	1.13	2.16	3.45	2.56	1.80	7.04	1.63	1.62	1.47	1.95	10.18	9.17	9.95
Roots	1.16	0.67	0.70	0.96	0.88	0.66	0.74	0.89	0.78	1.87	1.81	1.94	10.60	11.56	12.00
Entire plant	1.32	1.00	0.98	1.68	2.80	3.00	0.90	2.64	1.12	2.02	1.49	1.91	8.20	8.76	8.74

parable. In the case of the more intense light conditions (crop 2), sucrose and starch of the primary leaves of the final controls tend to be transformed into insoluble acid-hydrolyzable substances. Leaves of the treated plants are higher in sucrose and insoluble acid-hydrolyzable substances instead of high in starch.

In the case of higher light conditions (crop 2), starch and insoluble acid-hydrolyzable substances of the final control stems are higher, while sucrose is lower. The stems of the treated plants are higher in insoluble acid-hydrolyzable and water-soluble acid-hydrolyzable substances in place of the other fractions.

The roots of the crops showed no significant differences in chemical composition, except that in each case the acid-hydrolyzable fraction was higher in the treated plants as compared with the final controls.

If the stimulating effect of the indoleacetic acid increases the photosynthetic activity of the leaves, then despite the increased translocation of sugar to the stem end, there is an accumulation of sucrose in the leaves of the treated plants as compared with the controls. The fact that the stimulus is absent from the control plants probably accounts for the conversion of soluble carbohydrates to starch and insoluble acid-hydrolyzable substances. But in the stem of the treated plants all the carbohydrates are low except the insoluble acid-hydrolyzable materials. Thus, in the treated plants, it seems evident that there is translocation of carbyhydrates to the stem end.

The water-soluble acid-hydrolyzable substances are thought to consist mainly of pectins and gums (**5**). The insoluble acid-hydrolyzable material is considered to be composed of pectic substances (**9**), insoluble pentosans, protopectins, galactans, polymers of uronic acid, and a portion of the cellulose (**5**). Assuming compounds of the above types to be present, indole-3-acetic acid apparently produces a condition that causes soluble carbohydrates to move to the region of application and there to be synthesized into the higher polysaccharides. The previously-mentioned anatomical studies reported great meristematic activity, with lignin and secondary thickenings being laid down in the pith, phloem, endodermis, cambium, and other tissues. This chemical study agrees with those findings.

A greater percentage of the dry weight is accounted for in the final controls than in the treated plants. This suggests further that cellulose is a likely component of the remainder of the residual dry weight in the case of the treated plants.

Energy for growth is supplied through respiratory processes. Soluble sugars are low at the points of stimulated growth, and respiration as well as condensation may account for this fact. Examination of figures 4 and 5 shows that in crop 2, for which seemingly more favorable growth conditions

prevailed and greater growth occurred, the soluble respirable sugars are lower than in crop 1.

Summary

1. The stem ends of bean plants were removed and treated with two per cent. indole-3-acetic acid in lanolin. Tumors and roots appeared at the point of treatment.

2. Reducing sugars, sucrose, starch and dextrin, water-soluble acid-hydrolyzable substances, and insoluble acid-hydrolyzable materials were determined.

3. Dry weight was greater in the final control plants than in the treated plants.

4. There was a translocation of carbohydrates toward the point of treatment. The final controls differed from the treated in having a greater percentage of starch.

5. Stimulation by indole-3-acetic acid brought about conditions causing simple carbohydrates to be condensed to complex polysaccharides at the point of application.

6. Losses in dry weight of the treated plants indicated respiratory increases. Reduction of soluble carbohydrates in the treated stem ends was caused by increases in acid-hydrolyzable substances, and probably also by respiration incident to stimulated metabolic activity of the tumor.

The writer wishes to acknowledge his appreciation to Dr. J. W. MITCHELL for suggesting this problem, and to Professors C. A. SHULL and S. V. EATON for encouragement and suggestions.

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THE MIRSKY–PAULING THEORY OF THE STRUCTURE OF NATIVE, DENATURED, AND COAGULATED PROTEINS, AND SOME THEORETICAL ASPECTS OF THE EVOLUTION OF OXYGEN FROM THE IRRADIATED GREEN PLANT[1]

O. L. INMAN

This paper calls attention to some possible relationships which may exist between the sensitization and denaturation of proteins and one phase of the mechanism of photosynthesis.

MIRSKY and PAULING (5) point out that the most significant change that occurs in denaturation of a native protein is the loss of certain highly specific properties. The specific property here considered in the case of the process of photosynthesis is the possible denaturation of a protein which brings about the inhibition of the evolution of oxygen from the green plant when irradiated.

It has been shown by INMAN (3) that coagulation of the triturate of leaf tissue by pH, heat, calcium salts, dehydration, surface action, alcohol, etc., inhibits the evolution of oxygen. With pH held below 4.1 or above 8.1 for an hour at about 20° C., there was little indication of reversibility. The action of the combination of heat and dehydration is most interesting. If a whole leaf of *Trifolium repens* is dried in an oven at 30° C. for 48 hours, it may be powdered or used whole and allowed to absorb tap water, after which it will evolve oxygen upon irradiation. If, on the other hand, it is heated 48 hours at 45° C., it will not upon being permitted to absorb tap water evolve oxygen with irradiation. If the leaf, dried slowly at 30° C., is heated to 60° C. for a short time while dry it will still evolve oxygen. When leaves are triturated while fresh and dried at 30° C. for 48 hours, they will no longer evolve oxygen. A few plants such as *Nostoc* will stand extended drying at room temperature and still evolve oxygen upon adding water and visible radiation.

Freezing and thawing 57 times with carbon dioxide did not inactivate the leaf triturate of *Trifolium repens*. Immersion of the meal in liquid air for 30 minutes failed to inactivate it. In fact, the lower the temperature the slower the inhibition of the power to evolve oxygen. The lower alcohols are strong inhibiting agents. Studies of rates of inhibition and effects of surface as influenced by a variety of reagents indicate that the release of oxygen by the plant when irradiated is a chemical reaction, probably influenced by at least one enzyme. MIRSKY points out that denaturation of a protein is a

[1] Contribution from the C. F. Kettering Foundation for the study of chlorophyll and photosynthesis.

definite chemical reaction and not a vague disintegration of the protein, since a structural change in the molecule takes place. The action of crude or crystalline trypsin as an inhibitor for the evolution of O_2, caused either by the coagulation effect or by proteolytic action, seems to point to the denaturation of some rather specific native protein or protein group in the leaf triturate. This specificity is noteworthy owing to the fact that pepsin shows no inactivation. No recovery has been noted after inactivation by the use of trypsin. Boiled trypsin has no inhibiting action.

The triturated meal, upon standing in air or on being heated, becomes inactivated. The rate of inactivation is accelerated by a rise in temperature. This seems to indicate a chemical reaction, but does not specifically indicate whether a protein is being denatured. The hydrogen ion concentration inactivation curve has the same form as an enzyme hydrogen ion concentration curve. This indicates an enzyme reaction and the enzyme may be protein in nature. Also, the fact that leaves may be heated to a higher temperature when dry than when moist before inactivation occurs, points to a fact that fits in with the finding that some proteins behave as if there is coagulation in the case of freezing or dehydration, but no denaturation, or the molecules do not change in structure. There is, of course, the possibility of reversible denaturation in such a case.

The fact that shaking with fine glass beads inhibits the evolution of oxygen fits with the inactivation of some proteins by surface phenomena.

The proof of the existence of a "Blackman reaction" as well as a photochemical phase in the reactions of carbon fixation by the green plant is not out of line with the idea that a protein may participate in the mechanism as a part of the Blackman or the photochemical phase.

On the one hand, if the chlorophyll pigment as we know it in the pure form is associated with a protein in chemical combination, either the same protein exists in most green plants, or there is a specific active group common to many proteins, or there are a number of proteins to which the chlorophyll may be combined, or there are a number of chlorophylls in green plants. If, on the other hand, proteins merely act as carriers without sufficient bonding strength to enable one to extract the prosthetic group and the proteins together, or if the proteins merely give surface to the chlorophyll pigment, then coagulation with denaturation of the proteins could just as well serve as an inhibiting factor in the release of oxygen. The same may be true if the carbon dioxide, which is the most probable source of the free oxygen, is bound chemically with or absorbed on a protein which would be denatured by various treatments. While convincing experimental evidence is not at hand, it seems that chlorophyll is most probably to be considered as a kind of "prosthetic group" which when in the green plant is combined with an associated carrier. The work by KUHN and RUDY (**4**) in which a catalyti-

cally active flavoprotein was formed is most suggestive and from the biological standpoint provides a method of attack which offers much promise. The work of STERN and SALOMON (**8**) on ovoverdin suggests other possible applications to chlorophyll studies. FRENCH (**2**) considers a pigmented cell extract obtained from *Streptococcus varians* by high frequency sound waves to be a chromoprotein. There is certainly some reason to conceive of combining (chemically or by adsorption) extracted chlorophyll, which is photosensitive, with a carrier which can be sensitized and through this arrangement of radiation, prosthetic and carrier group account for the absorption and reduction of CO_2.

A similar system such as is postulated for the visual purple mechanism is not so fantastic even though speculative. Native visual purple $\underset{\text{dark}}{\overset{\text{light}}{\rightleftharpoons}}$ denatured visual purple (visual yellow) $\rightleftharpoons$ denatured protein retinene. If there is such a protein sensitization by some substance similar to retinene (in this case, perhaps chlorophyll or carotenoids), the protein or protein complex probably has a higher energy of activation than that of the visual purple mechanism but a lower energy of activation than most proteins.

The most serious difficulties apparent here are the lack of the true chemical structure of chlorophyll, especially as found in the cell, and the ignorance of the cellular metabolic reactions certainly necessary before the final reactions of the mechanism can take place. It is quite probable that studies of rates of reaction as measured by the evolution of O_2 or absorption of CO_2 are only the final steps in a long series of chain reactions. That the photosynthetic mechanism is a part of some of the basic physical and chemical reactions within the living cell is hard to refute with the evidence available.

While much work has been done which has led a number of workers to conclude that the enzyme catalase, a protohematin protein-complex, plays a part in the release of oxygen from some organic peroxide, this particular part of the mechanism seems to lack sufficient proof and may be too simple. EMERSON and GREEN (**1**) have recently reported work which failed to lend support to such a concept. From the work of MOLISCH (**6**) and INMAN (**3**), there is much more conclusive evidence that there is an enzyme (probably a native protein-complex) participating in the mechanism of photosynthesis but whether this has anything to do with catalase or an organic peroxide is still not clear. Some of the data on the evolution of oxygen, especially with dehydration at 30° C. fit in with MIRSKY's and PAULING's conception of coagulation and denaturation.

The probability of protein sensitization playing a part in some phase of the photosynthetic reactions is not to be dismissed without much further study. If some organic molecular complex somewhat similar to the visual

purple mechanism is involved along with the chlorophyll this would render the task of the reduction of carbon dioxide *in vitro* even more precarious until some agent can be found which will combine chemically with carbon dioxide or absorb it in such a manner that it becomes activated by visible light (see SPOEHR, **7**). The same fact might be true for the water molecule. There seems no good reason to assume that visible radiation directly activates either carbon dioxide or water since they do not absorb to any extent in the visible region of the spectrum. However, it is certainly possible that water or carbon dioxide or a combination of the two, when associated with chlorophyll which absorbs visible radiation, might become activated and enter into chemical and photochemical reactions one of which could be the release of oxygen.

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BRIEF PAPERS

HERITABLE VARIATIONS IN CHLOROPHYLL

J. C. IRELAND

(WITH ONE PLATE)

A spectrographic record of chlorophyll extracts is offered to replace the uncertain terms used to describe the degrees of greenness of leaves of grain sorghums in making inheritance studies. Chlorophyll deficiencies are described as albino, virescent, golden, mottled, striped, and with other words implying heritable characteristics (**2**). It has been found that some varieties of kafir after several generations of inbreeding have a percentage of virescent seedlings. Later developments of the plants show that many of them have an increase of concentration of chlorophyll over the normal plant and that very definite bands appear in spectrographic records. Continued sampling throughout the entire growing period during the last three seasons has revealed some interesting variations in the different strains.

Methods

From June to October, each Monday at 9 A. M., collections were made from ten head-to-row plantings. One-gram samples were weighed and ground in coarse sand. The small amounts of leaf material were used to secure uniformity during the early growing period, because seedlings were obtained at a very early stage. After grinding, 20 cc. of 95 per cent. alcohol was added to extract the chlorophyll, and the mixture was filtered after 4 hours. Ordinary glass test tubes were used as containers, but the tubes were marked and the samples were filtered into their respective tubes each week. Photronic cell readings (**3**) of the solution were made to determine the density and to make possible a comparison with GUTHRIE'S (**1**) standard solution.

A grating spectrograph was used, with a no. 1 photoflood bulb mounted in a microscope lamp stand as the source of light. The photoflood lamp provides a fairly uniform source of white light. Dufaycolor film was used to record the color, and it was processed immediately after the exposures were completed, following the directions provided by the manufacturer of the film. Exposures of the film were made for thirty seconds for each sample. The first exposure was that of distilled water, followed by GUTHRIE'S solution, then the ten chlorophyll samples. This routine was followed each week during the seasons.

A Zeiss hand sugar refractometer was used to determine the sugar concentrations after the nodes appeared and sugar became sufficiently concentrated to make readings. The juice expressed upon the plate of the refrac-

tometer from the fresh pulp represents a comparatively accurate estimate of the sucrose in the sap.

Results

Plate IV indicates the results of 1936 and 1937 spectographic records. Table I lists the various selections of sorghums used in the determinations of the seasons.

TABLE I

SORGHUM VARIETIES
CHLOROPHYLL AND SUGAR CONTENT
1936–1937

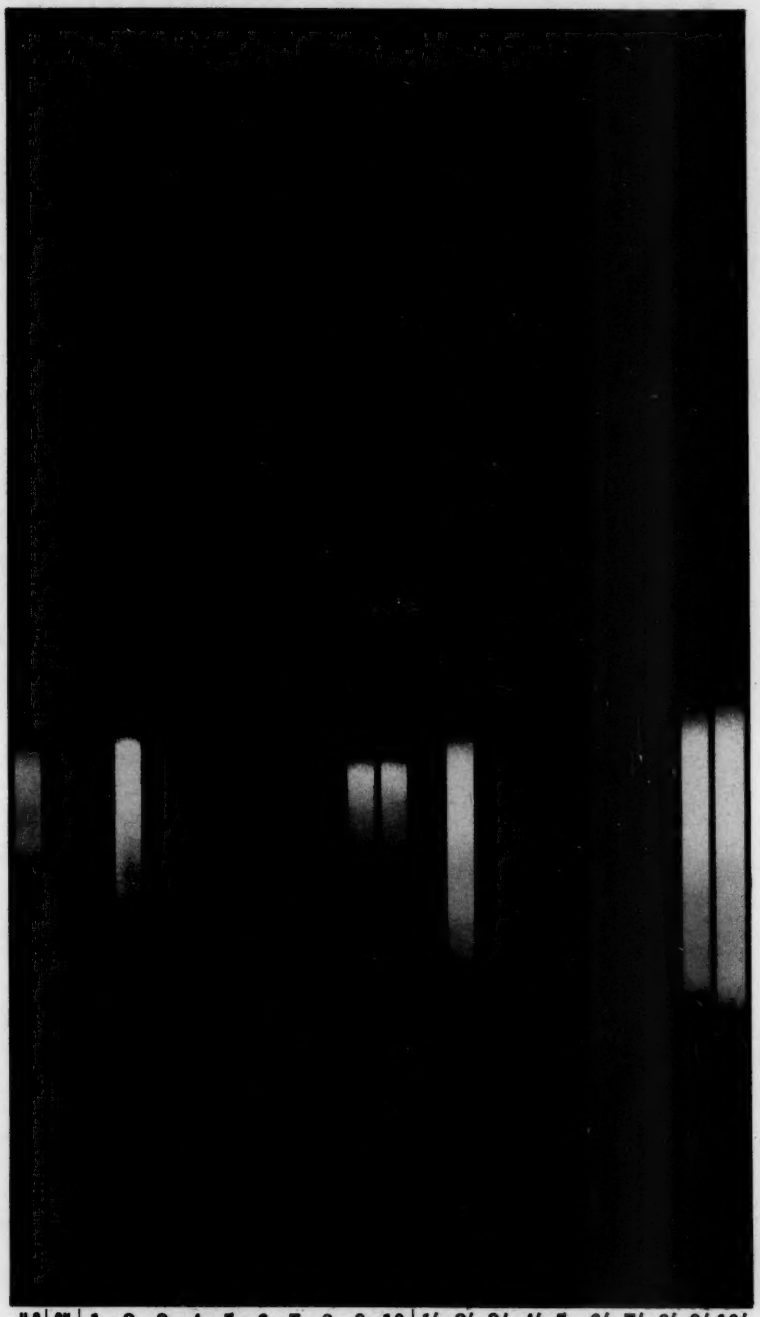
PLATE I SPECTROGRAPHIC NO.	VARIETY OF HYBRID	1936		1937	
		CHLOROPHYLL PHOTRONIC	SUGAR PER CENT.	CHLOROPHYLL PHOTRONIC	SUGAR PER CENT.
H_2O	Distilled water	00.0	00.0	00.0	00.0
Ck	Guthrie's solution	12.8	00.0	10.5	00.0
1 and 1′	Blackhull X Red Darso	13.3	12.2	10.6	9.9
2 and 2′	Reed X Milo	12.7	11.3	10.4	11.3
3 and 3′	Blackhull X Japonica	14.6	12.2	11.1	10.2
4 and 4′	Red Darso X Blackhull	14.1	11.1	10.6	10.5
5 and 5′	Pearl	15.9	11.3	11.2	11.5
6 and 6′	Red Kafir X Darso	14.8	11.2	10.5	9.9
7 and 7′	Red Kafir X Darso	14.0	11.9	10.6	9.1
8 and 8′	Blackhull X Japonica	12.9	12.3	10.1	13.1
9 and 9′	Blackhull	13.3	12.1	10.4	9.6
10 and 10′	Black Amber X Durra	13.8	12.9	10.7	7.5

It may be observed that the 1936 concentrations are generally higher than those of 1937, as indicated by the photronic cell readings. The spectrographic records of rows 2 and 2′, 9 and 9′, and 10 and 10′ do not seem to be determined by the intensities of chlorophyll. Rows 5 and 5′ indicate a

EXPLANATION OF PLATE IV

SPECTROGRAPHIC COMPARISON OF CHLOROPHYLL EXTRACTS

H_2O ..... Distilled water in test tube.
Check ..... GUTHRIE'S solution.
1 and 1′ ..... Blackhull X Red Darso.
2 and 2′ ..... Reed X Milo.
3 and 3′ ..... Blackhull X Japonica.
4 and 4′ ..... Red Darso X Blackhull.
5 and 5′ ..... Pearl.
6 and 6′ ..... Red Kafir X Darso.
7 and 7′ ..... Red Kafir X Darso.
8 and 8′ ..... Blackhull X Japonica.
9 and 9′ ..... Blackhull.
10 and 10′ ..... Black Amber X Durra.



IRELAND: CHLOROPHYLL

considerably higher concentration and lack the yellow and red extensions in the spectrographic record. Since these bands are duplicated in successive generations, regardless of concentrations, it may be assumed that they are due to heritable characters.

It may be observed that the chlorophyll and sugar values for 1937 are lower than those of 1936. The chlorophyll readings with the same photronic cell may be lower because of some deterioration of the cell, but the sugar differences are probably caused by variations in the supply of moisture.

Summary

The spectrographic variations in the chlorophyll extracts of grain sorghums are continuous throughout a season and in two generations. The concentration of chlorophyll and of sugars does not correlate with the widths of spectrographic bands.

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NICOTINIC ACID AND THE GROWTH OF ISOLATED PEA EMBRYOS[1]

JAMES BONNER

In earlier publications (**2, 3**) it has been shown that a number of different chemical substances function as "growth factors" for the isolated pea embryo. These accessory growth factors or their precursors are normally stored in the cotyledons whence they are mobilized by the seedling plant after germination of the seed. For extensive growth *in vitro* of the "cotylectomized" embryo, however, these growth factors as well as the ordinary foods must be supplied in the nutrient medium. By the use of a technique for the *in vitro* cultivation of isolated pea embryos in the dark, it has thus been shown that vitamin B_1, ascorbic acid, pantothenic acid, and theelin, all function as accessory growth factors for the seedling plant (**2, 3**). Evidence will be presented here which indicates that nicotinic acid, known to be a

[1] Report of work carried out with the aid of the Works Progress Administration, Official Project no. 465–03–3–342, Work Project no. N–9199.

component of the vitamin B complex and to be indispensable to the growth of certain microorganisms (**5**), is also a growth factor for the pea seedling.

Peas of the variety Perfection were sterilized in 0.1 per cent. $HgCl_2$ and were then soaked in sterile water for 6 hours. At the end of this time the embryos were cut from the cotyledons and were transferred to 50-cc. Erlenmeyer flasks containing 15 cc. of the pea nutrient medium described elsewhere (**1, 2**) which contains, in addition to inorganic salts, only 4 per cent. sucrose and 1 per cent. agar. All of the operations were, of course, carried out under sterile conditions and the few cultures which subsequently showed infection were discarded. To the basic nutrient medium was added the desired amount of accessory growth factor, in this case nicotinic acid or vitamin B_1, or both. The cultures were kept in a controlled dark room at 24° C. and the shoot length of each plant was recorded weekly for 4 weeks.

Table I gives the result of two parallel experiments which were closely comparable and which have been combined in this table.

TABLE I

EFFECT OF NICOTINIC ACID AND OF VITAMIN B_1 ON THE SHOOT GROWTH OF ISOLATED PEA EMBRYOS

ADDED GROWTH FACTOR	NUMBER OF EMBRYOS GROWN	SHOOT LENGTH AFTER			
		1 WEEK	2 WEEKS	3 WEEKS	4 WEEKS
		mm.	*mm.*	*mm.*	*mm.*
None (control)	40	15	22	26	28
Nicotinic acid 0.01 mg./flask	40	17	25	32	37
Vitamin B_1, 0.01 mg./flask	40	22	32	40	49
Nicotinic acid + vitamin B_1, each 0.01 mg./flask	40	23	34	50	68

The control embryos to which no accessory growth factor is added are able nevertheless to grow somewhat under these conditions. This is undoubtedly to be attributed in part to the growth factors which the embryo contains as it is isolated from the cotyledons, and in part to a limited capacity of the embryo to synthesize some growth factors from the constituents of the basic medium (**3**). If 0.01 mg. of nicotinic acid per culture flask is added to the medium, the growth of the shoot is somewhat accelerated, and after 4 weeks the plants supplied with nicotinic acid are one third taller than the controls. Vitamin B_1 at the same concentration in the medium exerts a considerably larger effect upon the growth of the shoot, and the further addition of nicotinic acid, as shown in the last line of table I, further enhances this effect.

It is now well established that vitamin B_1 in its rôle as a plant growth factor influences primarily the root (**1, 2**), and the effect of this substance upon the growth of the shoot is probably then the result of its influence on the root system. It is of interest therefore to determine what influence nicotinic acid exerts on the growth of the root. This was investigated by means of experiments in which nicotinic acid was added to the nutrient medium in which isolated pea roots were grown *in vitro*.

Tips were cut from the roots of seedling peas and were grown for one week *in vitro* in order to deplete them of their initial supply of vitamin B_1. At the end of the week, fresh tips 1 cm. long were cut from these roots and transferred to fresh medium with added growth factors as shown in table II.

TABLE II

EFFECT OF NICOTINIC ACID AND OF VITAMIN B_1 ON THE GROWTH OF ISOLATED PEA ROOTS

ADDED GROWTH FACTOR	NUMBER OF ROOTS GROWN	TOTAL LENGTH AFTER 1 WEEK
		mm.
None (control)	40	21 ± 0.5
Nicotinic acid 0.01 mg./15 cc.	40	21 ± 0.6
Vitamin B_1 0.01 mg./15 cc.	40	48 ± 1.1
Nicotinic acid + vitamin B_1, each 0.01 mg./15 cc.	40	56 ± 1.0

It may be seen from table II that nicotinic acid alone exerts no effect on the growth of the isolated pea root. It does, however, increase somewhat the response of the root to vitamin B_1. It may be, therefore, that the effects of nicotinic acid upon the growth of the shoot, as shown in table I, are primarily due to effects of this substance upon the root itself.

Nicotinic acid and/or closely related derivatives are known to occur widely in natural products: in leaves, seeds, yeast, etc. (**4**). It might be suspected *a priori* that this general distribution indicates a function of nicotinic acid in higher plants. From the evidence presented in this paper it may be tentatively concluded that this is indeed the case.

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EFFECTS OF FLUE DUST ON THE GROWTH OF SUNFLOWER AND GOLDEN WAX BEAN

BARNEY BARNETT COHEN

Investigations concerning the influence of flue dust on plant growth have been few and in most cases reported by foreign investigators. In 1915 the Journal of the Society of Chemical Industry (**5**) reports an analysis of flue dust for possible injurious substances and concludes that none were found. VÜRTHEN (**11**) in analyzing samples of dust from the Kaliasch Iron Works thought that the average percentage of chlorides present was sufficient to reduce the fertilizing value of flue dust. VOELCKER (**9**) suggested to purchasers that guarantees be demanded not only for the potassium present but for the total *soluble* potassium. He also reports (**10**) in experiments with wheat plants that not only the percentage of Fe present was to be considered, but also whether the Fe was present in ferrous or ferric state. VLASYAK (**8**) studied the effects of Fe-Mn flue dust and found that dusts with not more than 4 per cent. alkalies are suitable fertilizers for beets.

In the experiment reported here, flue dust from the Wisconsin Steel Mills, Chicago, was used in varying proportions in the soil. Sunflowers and Golden Wax beans were grown in these soils which contained varying proportions of flue dust and observations and records were made of growth behavior. The analysis of the flue dust, supplied by the Wisconsin Steel Mills, is as follows:

Fe	50.34	per cent.
C	11.53	" "
Si	10.83	" "
CaO	3.20	" "
Al_2O_3	2.76	" "
Mg	0.67	" "
MgO	0.41	" "
P	0.246	" "

Procedures

The sunflowers and Golden Wax beans were grown in glazed pots, six being used for each test. One pot, with soil only, was used as a control. The second contained a mixture of one thousand parts of soil to one part of dust; the third, ten parts of soil to one part of dust; the fourth, one part of soil to one part of dust; the fifth, one part of soil to three parts of dust; and the last flue dust alone. The above proportions of dust and soil were thoroughly mixed before being placed in the pots. The plants were watered at two day intervals with tap water, and, observations were recorded from day to day.

Dry and green weights of the sunflower (table I), and number and weights of the fruits of the bean (table II) were recorded. Typical results have been selected as a basis for comparison.

Data and results

TABLE I

DRY AND GREEN WEIGHTS OF SUNFLOWER

SOIL USED		SOIL ONLY	1000:1	10:1	1:1	1:3	DUST ONLY
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Dry weight	Top	23.00	31.00	14.9	12.00	5.00	0.84
	Root	2.44	3.26	1.02	.83	.351	0.091
	Total	25.44	34.26	15.92	12.83	5.351	0.931
Green weight	Top	129.0	180.2	124.0	112.3	34.0	6.100
	Root	10.2	21.0	7.3	5.7	3.1	.930
	Total	139.2	201.2	131.3	118.0	37.1	7.03

TABLE II

YIELD OF GOLDEN WAX BEEN FRUITS

	SOIL ONLY	1000:1	10:1	1:1	1:3	DUST ONLY
Number of fruits	24	23	21	18	10	None
Weight, grams	36.15	36	20.3	21.1	11.1	.....

Results obtained with Golden Wax bean indicate that in excess of 10 per cent. flue dust, anomalous growth effects were noticeable, and became more pronounced with increasing concentrations. Chlorosis of the lower leaves, stunting, delayed flowering, and reduced size and number of fruits were some of the observed results of disturbed metabolism. The growth of the Golden Wax bean in complete flue dust was very meager.

With the sunflower, results of a different nature were obtained. In the mixture of one part dust to one-thousand parts of soil, which is comparable

to additions of fertilizers, increased yields were obtained. As in the Golden Wax bean, concentrations in excess of 10 per cent. showed decreased growth and chlorosis.

Discussion

The induced chlorosis may be accounted for by two factors: first, the excess of Fe present and, second, the alkalinity of the soil. MARSH and SHIVE (**6**) believe that the concentration of soluble Fe above the optimum may result in a condition in which the plant is unable to use the Fe present and so suffers from deficiency even in the presence of extremely high Fe concentrations.

Of course, the possibility of deposition of insoluble Fe in stem and root tissues might cause failure of translocation of Fe within the plant. This seems improbable, however, since microchemical tests (**7**) have shown Fe to be present in upper and lower parts of the bean stem.

The high alkalinity of the flue dust is probably an important factor in inducing iron chlorosis. The results of 4 tests showed pH values of 7.9, 7.9, 8.0, and 7.9. Experiments conducted by JONES (**3**) and JONES and SHIVE (**4**) indicate that the decrease of pH from 4.8 to 4.2 increased the availability of Fe when supplied in the form of ferric phosphate.

HOPKINS and WANN (**1, 2**) have observed also that in *Chlorella* Fe was unavailable at high pH values.

The increased growth of the sunflower in the second pot indicates that this plant is able to utilize the abundance of essential elements provided in the flue dust and at the same time tolerates the high pH value resulting from its application in quantity.

Conclusions

1. Flue dust, in concentrations in the soil greater than 10 per cent., has a decided effect in reducing the yield of fruits of the Golden Wax bean.

2. In concentrations in the soil ranging near 0.1 per cent. it has a decidedly favorable effect on the growth of the sunflower.

3. In concentration in the soil above 10 per cent., it has a depressing effect upon the growth and yield of the sunflower.

4. Sunflower and Golden Wax bean grown in complete flue dust show meager growth, the effect being more pronounced in the case of the bean.

The writer wishes to express his appreciation to Dr. C. A. SHULL for suggestions in connection with this problem.

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EFFECT OF SUN AND SHADE ON PIGMENT DEVELOPMENT

WILLIAM A. BECK

In a preliminary experiment which preceded a study of seasonal variations of pigment production in field plants, the writer obtained interesting results regarding the effect of sun and shade on the production of pigments in plants.

The work was done in the first week of June, 1936. It was a sunny season and the plants were vigorous. Plantain (*Plantago major* L.) was chosen because specimens could be found in sunny and shady places not far removed from each other. The sun plants were taken near the top of a hill where the slope was slight, and the shade plants were taken from the woods on the same hill where the slope was about the same. Direct sunlight never struck the shade plants, but diffuse light was plentiful throughout the day. The plants in both places had not been disturbed or mutilated from the time of early Spring. The four youngest leaves were chosen for tests in both cases so that the leaves were comparable with respect to the age factor.

It was previously reported by JÖNSSON, LUBIMENKO, STÅLFELT, ROSÉ,

LUNDERGÅRDH, and URSPRUNG that more chlorophyll develops in plants that are shaded than in those that grow in sunny places. Nothing is stated in the literature, so far as the writer's knowledge goes, about the relative amounts of xanthophyll and carotene which develop in direct sun light and in the shade. In the experiments reported here, simultaneous determinations of all three pigments were made for the plants growing in direct sunlight and in the shade.

The comparison of the amount of pigment yielded by plants growing in the two places was made on the basis of leaf area, wet weight, and dry pulp weight. The results obtained by the area method were almost identical with those obtained with the method employing the weight of the dry pulp. Both were considerably different from the results obtained with the wet weight method because the plants growing in direct sunlight contained considerably more water than did the shade plants.

The wet weight per cm.^{2} of leaf area was 18.1 mg. for the shade plants and 22.8 mg. for the sun plants. The weight of the dry pulp for shade plants and sun plants was the same, *i.e.*, 3.0 mg. per cm.^{2} of leaf area.

The sun plants contained 0.025 mg. of chlorophyll per cm.^{2} of leaf area, and the shade plants 0.035 mg. The shade plants contained 40 per cent. more chlorophyll. The sun plants contained 0.0044 mg. of xanthophyll per cm.^{2} of leaf area, and the shade plants only 0.0038 mg. The shade plants contained 13.6 per cent. less xanthophyll than the sun plants. The sun plants contained 0.00081 mg. of carotene per cm.^{2} of leaf area and the shade plants 0.000828 mg. The shade plants contained slightly more carotene than the sun plants.

The results of the experiment show that direct sunlight favors the storage of water in the plantain, which reminds one of the water storage by succulent plants. The development of chlorophyll is considerably favored by shaded conditions; the same conditions slightly favor the development of carotene. The development of xanthophyll is slightly favored by sunny conditions.

INSTITUTUM DIVI THOMAE
CINCINNATI, OHIO

TEACHING METHODS[1] IN PLANT PHYSIOLOGY

CHARLES J. LYON

At the annual meeting of the American Society of Plant Physiologists held at Indianapolis in December, 1937, the programs closed with a symposium on teaching methods in plant physiology. The chairman, Dr. CHARLES A. SHULL, of Chicago, introduced the speakers and led the discussion after the papers had been presented. Prepared papers were read by Dr. OTIS F. CURTIS, of Cornell, Dr. F. G. GUSTAFSON, of Michigan, and Dr. B. S. MEYER, of Ohio, all teachers of large classes in large universities, and Dr. CHARLES J. LYON, of Dartmouth, who has small classes in an Arts College. The good attendance indicated a real interest in the seminar because it was scheduled as the last session of the society of plant physiologists, with everybody tired.

Each of the four speakers introduced by the chairman used about twenty minutes to explain the ideals and procedure by which plant physiology is taught at the institutions represented. Of the three objectives we outlined at Kingston, the information angle was assumed and the scientific attitude was emphasized only as the speakers showed how it is encouraged by oral discussions that promote accurate thought and logical reasoning. Each teacher described the mechanics of his course, the nature of the students and the level at which the topics are treated in the general, elementary course. There was marked agreement in most respects. The greatest differences appeared between the college and the university, and in the emphasis which individual teachers place upon such points as write-up of experiments, the preparation of solutions, the use of text assignments, and the critical interpretation of experimental results. No one claimed that his classes performed accurate, quantitative experiments, dipped far into chemistry, or covered the field of plant physiology. Semester courses were the rule, with the understanding that future plant physiologists need further training in advanced courses.

Some of the practices mentioned by the speakers are indicative of current trends. At Michigan, Dr. F. G. GUSTAFSON has a class of 25 to 40 with a mixture of forestry, horticulture, and Ph.D. interests. The course has a schedule of 8 hours per week that allows him to start with growth and have the students carry their own plants throughout the semester. The laboratory experiments are written up in full, but the results are also used for unannounced group discussions during laboratory hours. At Ohio State, Dr. MEYER has a class that runs very large—100 or more—for the two-quarter course (22 weeks). The students have a great variety of interests, with

[1] Abbreviated from a report to the New England Section at Orono, Maine, May 13, 1938.

agriculture predominant. The course is of necessity carefully planned about a series of selected assignments, lectures, and group discussions at crucial moments, and the printed laboratory manual with which you are familiar.

The story of a class of 6 or 8 Dartmouth men, handled by a modified tutorial system, contrasted with the accounts for Ohio and Michigan before, and with that for Cornell to follow. Emphasis was placed on the shortage of enthusiasm for a complex subject like ours, the total lack of a supporting graduate or professional training school, and the usual difficulties in providing the expensive quarters and equipment for a relatively unimportant elective course in a biology department. The general procedure of lecture *following* an organized but informal laboratory study of a topic was distinctive because it was so unlike the methods described for university courses. Oral examinations outside of class hours were mentioned as another instance of special devices that are possible in small classes.

At Cornell the semester course is taken by 50 to 70 students in laboratory sections that are limited to 24. In spite of the large enrollment and the need for a highly organized procedure, Dr. CURTIS emphasizes the scientific method and the critical interpretation of experimental evidence. Class experiments are limited to simple combinations of plants and apparatus, designed or selected to make the student learn a maximum number of principles with a minimum number of laboratory hours. Probably all teachers would claim the same objective but from all available evidence it appears that Dr. CURTIS has given the point rather more attention than other teachers whose methods have been described in detail.

The one method emphasized by the three university teachers and used in different form by the other speaker at Indianapolis, is the problem question as the basis of class discussion. Dr. CURTIS, Dr. GUSTAFSON and Dr. MEYER pass out mimeographed sheets of questions and require the students to work out the answers. Many of the questions are only the usual review questions, such as:

What conditions affect stomatal movement? or,
What are the differences between fermentation and respiration?

The other type of question is considered more nearly ideal for teaching students useful plant physiology. Here are a few examples:

What occupies the space between the protoplasm and the cell wall when a cell is plasmolyzed by a sucrose solution?

Cells on the convex side of a bending shoot were found to have a lower osmotic concentration, a lower turgor pressure and a higher suction tension than corresponding cells on the concave side of the same stem. What explanation can you offer?

In using a burette respirometer containing germinating seeds, what is measured by the change in level of the hydroxide solution?

If you wished to hasten the removal of carbohydrates from a given leaf, how could you do it?

Following a brief summary by Dr. SHULL, the open discussion of the evening was free and lively but it included a variety of unrelated matters and most of the questions were left unsettled. They included: definition of osmotic pressure; level *vs.* content in elementary courses; value of having the student grow experimental plants; use of fungi for experiments; and the possibility of students doing work in the nature of original research. Only the necessity of connecting with late trains stopped the session at this point; but for some reason the point of problem questions was not brought up again. To me this appeared to be the *most important single method described during the symposium.* Essentially it is the assignment of a topic for the student to explain.

As I have thought about it since, I am so impressed by its possibilities, its theoretical values, its adaptability to different institutions and also by its limitations, that I have suggested it to the officers of this section as a topic for the next symposium we conduct in the field of teaching methods.

DEPARTMENT OF BIOLOGY
DARTMOUTH COLLEGE

NOTES

Ottawa Meeting.—The summer meeting of the American Society of Plant Physiologists was held at Ottawa, Canada, June 28 to 30, 1938. Joint sessions were held with the American Phytopathological Society, and the Genetic Society of America. In the first of these joint meetings microelements and deficiency diseases were emphasized in studies of boron deficiency, spectrographic methods of detecting microelements, histological and cytological changes accompanying microelement deficiencies, and studies of thallium in relation to frenching. The symposium on microelements also considered the relation of trace elements, including iodine, copper, cobalt, manganese, and toxic levels of selenium and fluorine, to animal physiology.

In the second symposium, which occupied all of June 29, drought relations were the subject of discussion. The morphology and physiology of wheat plants grown with reduced water supply were given detailed consideration, and the similarities and differences between drought and frost resistance were discussed. Transpiration and lag in water absorption to meet the transpiration demand were emphasized, the lag being ascribed to resistance in the root tissues rather than in the stems. The water economy of trees in relation to drought also received attention, and the effect of cultivation on soil moisture relations. During the afternoon, methods of breeding drought resistant plants were reviewed, with special reference to wheat breeding and crosses between *Triticum* and *Agropyron,* and studies of the roots of wheat and other field crops. Apparatus for studying drought resistance under controlled conditions was described.

The Thursday morning session was given over to short reports of investigations of general interest. Assay methods for growth promoting substances, progress in the practical utilization of hormones, limiting concentrations of SO_2 for plant development, and the effect of SO_2 on the growth of conifers, apparatus for light, temperature and humidity control, and studies of cell permeability in relation to fungus parasites were among the topics presented.

On Tuesday afternoon a visit to the Central Experimental Farm at Ottawa was enjoyed by all of the plant science groups. In connection with this inspection trip, all visitors were guests of the Farm at a tea later in the afternoon. Thursday afternoon afforded opportunity to visit the laboratories of the National Research Council. Visitors were conducted through these laboratories by Dr. ROBERT NEWTON, Director of the Division of Biology and Agriculture.

On Wednesday, at noon, the society luncheon was held at the Chateau Laurier. Short talks were made by president O. F. CURTIS, vice-president

elect GEORGE W. SCARTH, and past presidents BURTON E. LIVINGSTON and W. E. TOTTINGHAM. Dr. ROBERT NEWTON served as toastmaster.

This was the eighth summer meeting, and was a very successful and satisfactory one, thanks to the efficient service of the program committee headed by GEORGE W. SCARTH, of McGill University. The reports of the secretary-treasurer, Dr. F. P. CULLINAN of the United States Department of Agriculture, showed healthy and steady progress in support.

Richmond Meeting.—The fifteenth annual meeting will be held at Richmond, Virginia, on December 28 to 30, 1938. The Hotel Jefferson has been designated as headquarters, and the meeting rooms for the sessions are located in the same hotel, a most satisfactory arrangement. The usual practice of holding the annual dinner on the first day, December 28, will be followed and the program committee requests that members and friends secure their tickets for the dinner promptly on arrival at the time of registration. The dinner is open to all who care to attend, and the announcement of the award of the CHARLES REID BARNES life membership, the HALES award, and the retiring president's address will make the occasion memorable. A session devoted to the problems of teaching plant physiology is planned for the closing hours on Friday.

Dedication.—This number of PLANT PHYSIOLOGY is dedicated to Professor FRANCIS ERNEST LLOYD in honor of his seventieth birthday anniversary on October 4, 1938, as a mark of our deep appreciation of his life-long service to plant physiology, and of his early service to the American Society of Plant Physiologists as president of the society. The following appreciation, prepared by Professor LLOYD's long-time colleague at McGill University, will find an echo in the hearts of everyone who has had the privilege of knowing Professor LLOYD and his important contributions to science. Professor LLOYD was awarded the second CHARLES REID BARNES life membership in the society at the Nashville meeting in 1927 in recognition of his valuable work.

Francis Ernest Lloyd.—The career of emeritus Professor F. E. LLOYD, who reached the age of 70 on October 4th, displays versatility and breadth of interest as well as an occasional radical change of outlook which may be compared to conversion. Indeed it was this experience in the orthodox sense of the term that sent LLOYD as a young man to Princeton to study for the ministry; but soon another change of heart turned his enthusiasm from theology to biology. In his earliest publications he appears as a biologist of the old school; catholicity of interest is reflected in the subjects dealt with, *viz.*, boring molluscs, bits of anatomy and taxonomy, and, in a more impor-

tant paper, the embryology of the Rubiaceae. Some glimmerings of a new light are seen in this last paper which gives to the anatomical facts a physiological explanation. But LLOYD'S road to Damascus only brought him to the crisis when he reached the Arizona desert. "Those were the days," the early days of the Tucson laboratory, the stimulation of its physical and mental atmosphere and the new vision which converted our old-fashioned botanist into an ultra-modern physiologist, one to whom teleology was anathema and mechanism an inspiring creed! *The Physiology of Stomata* published at this period is notable not only for its positive contributions to the knowledge of stomatal mechanism but also for the iconoclastic glee with which the teleological assumption of perfect regulation of transpiration by stomata is discredited. As was predicted by FRANCIS DARWIN at the time, this work proved highly stimulative and it is perhaps the most quoted of all LLOYD'S publications.

The new recruit to physiology was soon marching in the van of the movement, applying biophysics to the physiology of the cell. In this strain is his work on the fate of tannin during the ripening of fruits—not oxidation, he found, but adsorption by another colloid; and again on the growth of pollen tubes in acid and alkali which he showed to run parallel to the swelling of gelatin. The biophysical approach became the evangel of his teaching and the elementary course in General Physiology which he developed in later days at McGill was even then a pioneering effort.

Though a fundamentalist at heart LLOYD frequently entered the field of applied research. His monograph on Guayule and its methods of culture which formed the basis of later practical developments, and his correlation of boll-shedding of cotton with a sinking of the water table (under Alabama conditions, at least) are examples of direct attack on practical problems.

But his best work dealt with topics of which the interest is primarily academic, such as the conjugation of *Spirogyra*, especially the rôle of contractile vacuoles in the process, and the varied functions of carnivorous plants, especially the trap mechanism of *Utricularia*. It is subjects such as these that particularly suit LLOYD'S talent—subjects possessing a certain dramatic interest and requiring for their study infinite patience, technical dexterity, and keen observation. Though early in the biophysical field, his training was hardly such as to enable him to push that type of investigation to the utmost—much to his regret. He is wont to say that his only training was listening to the dictum of his first and much beloved biology teacher, Professor GEORGE ("Pat") MACLOSKIE of Princeton University: "Nature is the highest authority." This explains, perhaps, the seemingly erratic course which his researches have pursued. Ever questioning nature he followed whatever lead she gave him. He dipped wherever he saw a fish.

One ruling passion, however, has persisted through all the turns and twists of LLOYD'S career, an insatiable curiosity to discover how a piece of

mechanism works. The same desire "to see the wheels go round" that apprenticed LLOYD as a youngster to the watchmaking trade still burns strong in old age and infirmity as he probes the beautiful mechanism of the bladderwort trap. Nature is having her revenge! The youth who scoffed at the adaptations of stomata has lived to become the mature interpreter of one of the most perfect and puzzling adaptations in the plant kingdom. Perhaps a final conversion is due!

Not the least of LLOYD'S contributions to physiological science is the arousing of public interest through his lectures. Possessed of a strong dramatic sense, he could tell the story of his researches with effect; and artistically gifted, he illustrated his narrative with beautiful hand-coloured slides and motion pictures which are known the world around.

LLOYD is a cosmopolitan. He has carried on research and given his lectures in all parts of North America, many European countries and all the continents. Now he enjoys a busy rest at Carmel, California, working daily in the old coastal laboratory of the Carnegie Institution of Washington amid scenes and associations that recall the days when physiology claimed him for her own.—GEORGE W. SCARTH.

Errata.—A small list of errors in volume 13 of PLANT PHYSIOLOGY is printed at the close of the table of contents. It is advisable to record the corrections appropriately at the places where the errors occur, so as to avoid misinterpretations. The editor solicits the aid of all authors in detecting and correcting errors which occur in their papers. We do not have time to proof read the printed volumes, and appreciate the valuable aid extended by those who reported mistakes.

Minor Elements.—Because of the rapid exhaustion of previous editions of the *Bibliography of References to the Literature on the Minor Elements and Their Relation to Plant Nutrition,* we are making a pre-publication announcement of the early appearance of a third edition of this invaluable reference bibliography. It is to be issued in January, 1939, by the Chilean Nitrate Educational Bureau, Inc., 120 Broadway, New York. As long as the supply lasts copies will be available at $1.00 per copy. This edition will carry about 4700 abstracts and references, and it is expected to be the last complete edition the Bureau will undertake to publish. Members are urged to place orders promptly with Mr. HERBERT C. BREWER, Director, at the address given. Every member of the society should possess a personal copy of this annotated bibliography.

Outlines of Biochemistry.—A welcome addition to the literature of biochemistry is the second edition of Dr. R. A. GORTNER'S *Outlines of Biochem-*

istry, published by John Wiley and Sons. The work has been carefully rewritten, and chapters added dealing with oxidation-reduction, the flavins, and the hormones. It is brought up to date in such progressive fields as the physiological chemistry of the vitamins, sterols, and other hormones. Plant physiologists would profit by making this splendid volume a daily companion. It is listed at $6.00 per copy.

Chromosome Structure.—A new volume in the Protoplasma-Monographien series, no. 14, is Dr. LOTHAR GEITLER'S (University of Vienna) *Chromosomenbau.* It is a technical discussion of the structure of chromosomes, presented in six chapters. It deals not only with the external features of chromosome morphology, but also with the microscopic and submicroscopic structures. It is not intended for beginners, but for those already well versed in the knowledge of chromosomes, and their behavior in all kinds of cells. Cytologists and geneticists will find it valuable in bringing together and summarizing a large field. About 700 titles are included in the literature list. The work is well illustrated (69 figures) and is appropriately indexed. The quoted price, bound in red cloth uniform with other volumes in the series, is RM 15. Orders may be sent to Gebrüder Borntraeger, Berlin.

Leaf Xanthophylls.—An excellent monographic study of the leaf xanthophylls has been published by the Carnegie Institution of Washington as Publication no. 490. The author is Dr. HAROLD H. STRAIN, of the Institution.

The introduction gives an account of the methods of separation of the numerous xanthophylls from one another, the molecular structure of the carotenes and xanthophylls, nomenclature, physiological functions, state of the pigments in leaves, and their oxidation in killed leaves.

The following section deals with the principal properties of 13 carotenes and 30 xanthophylls. Three xanthophyll esters and 7 carotenoid acids are described, and a lone unclassified member, rhodovibrin.

The experimental techniques require great skill, and involve extraction, isolation and separation in pure form, and determination of the main properties, such as absorption spectra, melting points, optical properties, empirical formulae, color reactions, microscopic examination, and quantitative estimation of leaf xanthophyll.

The results of these examinations occupy the major portion of the monograph. Chromatographic adsorption has proved an invaluable aid in the separations. A frontispiece in color illustrates the method used in this work. The xanthophylls turn out to be mainly mono-, di-, tri-, and tetraoxy derivatives of carotenes, with lutein the main constituent in a mixture

of a dozen or more xanthophylls in any given sample. Crystallization from petroleum ether prevents the main losses incident to the WILLSTÄTTER and STOLL procedures. The entire monograph reflects the skill and patience with which the problems have been approached. The fact that only partial success has been attained, especially in the quantitative aspects of the work, emphasizes the difficulties encountered all along the line. The contribution is a very worthy one, and plant physiologists will be grateful for the insight which the publication gives into one of the difficult phases of plant biochemistry. A bibliography of 190 titles, and author and subject indexes make it useful in connection with past work. It deserves a place in the libraries of all who are interested in plant pigment chemistry.

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